201-15547

APPENDIX

Robust Summaries for Substances in The HPV Test Plan for the Polyol Esters Category of the Aliphatic Esters Chemicals

Part I. HPV Substances in the Polyol Esters Category
Part II. Surrogate Polyol Esters

August 24, 2004

Appendix -Robust Summaries for Aliphatic Esters - Polyol Esters HPV Test Plan

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Part I - Robust Summaries for HPV Substances in the Polyol Esters Category of Test Plan

HPV Polyol Esters Substances
Identified by CAS Numbers and as organized in Table 1B of the HPV Test Plan

Part II - Robust Summaries for Surrogate Polyol Esters

Four Surrogate Polyol Esters Substances

The four structurally analogous surrogate polyol esters are:

- TMP ester of heptanoic and octanoic acid (CAS No. 189120-64-7)
- Hexanedioic acid, mixed esters with C10-rich, C9-C11 alcohols and TMP (CAS No. 180788-27-6)
- Hexanedioic acid, mixed esters with heptanoic, octanoic and decanoic acid and PE (CAS 68130-55-2)
- Pentaerythritol esters of isooctanoic and C8-10 fatty acids (No CAS Number Assigned)

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Pentaerythritol esters of isooctanoic and C8-10 fatty acids
(No CAS Number assigned yet)

ROBUST SUMMARIES

PART I. HPV Substances in the Polyol Esters Category

Boiling Point (CAS No. 11138-60-6)

Test Substance	Decanoic acid, ester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol octanoate
CAS Number	11138-60-6
Remarks	Purity not indicated
Method/guideline	OECD 103; met physical/chemical testing for CEPA regulations
Test type	Boiling point (modified of Siwoloboff's method)
GLP	No
Year	1996
Procedure	The test substance (10 mm) was put above an air layer (2 mm) in a sealed glass Pasteur pipette and placed in a forced air oven at 305°C and 102 kPa.
Results /Remarks	Boiling point >300 °C at 102 kPa. Movement of test substance was <5 mm, no color change.
Conclusions	Boiling Point > 300 °C
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP. Not clear if modification of original of Siwoloboff method will have significant impact on accuracy since bp was greater than 300 °C and was not carried out at temperature above that temperature.
References	Unpublished confidential business information.
Other	Date last updated: December 3, 2003.

Vapor Pressure (CAS No. 11138-60-6)

Test Substance	Decanoic acid, ester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol octanoate
CAS Number	11138-60-6
Remarks	Purity not indicated
Method/guideline	OECD 104; met physical/chemical testing for CEPA regulations
Test type	Vapor pressure
GLP	No
Year	1996
Procedure	The isoteniscope method described in OECD 104 was used.
Results /Remarks	Temp. [°C] — Vapor Pressure [Pa] 20 — <13 25 — <13 50 — <13 100 — 40 150 — 267 200 — 1107 250 — 3466 300 — 6666 350 — 21998 375 — 58662

	The limit of determination (LOD) was 13 Pa.
Conclusions	Vapor pressure at 25°C was <13 Pa (limit of determination)
Remarks	The recommended range of vapor pressures using this method is 10^2-10^5 Pa according to OECD 104. The vapor pressure of test material at temperatures below 150°C lies below this level. At 350°C decomposition of test material was observed. So part of the increase in vapor pressure at temperatures 350 and 375°C could have been due to other compounds formed in the decomposition process. In the report is stated that above 670 Pa, the repeatability is ~10%. Below this level no information is available in the report. Since OECD 104 recommends this method for vapor pressures in the range 10^2-10^5 Pa also the value at 150°C is acceptable. Including also the decomposition of the test substance it can be concluded that in this test only values of vapor pressures between 150 and 300°C are reliable.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Vapor pressure value at 25°C was at limit of determination.
References	Unpublished confidential business information.
Other	Date last updated: December 3, 2003.

Partition Coefficient (CAS No. 11138-60-6)

Test Substance	Decanoic acid, ester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol octanoate
CAS Number	11138-60-6
Remarks	Purity not indicated
Method/guideline	OECD 107; met physical/chemical testing for CEPA regulations
Test type	Partition coefficient (kow)
GLP	No
Year	1996
Procedure	Mutually saturated n-octanol and ultrapure water were used in the test. The test was performed with 12 mL water and 6, 12 and 24 mL n-octanol; 300 µL of a solution of test material in acetonitrile (2.54 g/L) was added. A blank with 12 mL water and 12 mL n-octanol was included. After 21 min. of shaking (22°C), the solutions were centrifuged, the phases separated and analyzed by GC-FID. In the water layer filtration and extraction with methyl t-butyl ether (2 mL) preceded the analyses with GC-FID.
Results	The test material was not found in any of the aqueous phases, indicating that its concentration was less than the limit of detection of 0.3 µg/mL. Conc of test material in octanol and aqueous phases given below in table.

Amount solution (mL)		**Concentration (µg/mL)**			
Water	**Octanol**	**aq. phase**	**octanol phase**	**K_{ow}**	**log(K_{ow})**
12	12	<0.3	64	>213	>2.3
12	6	<0.3	139	>462	>2.7
12	24	<0.3	28	>92	>2.0

Conclusions	log(K_{ow}) >2.7 at 22°C K_{ow} values are minimal, as concentrations of test material in the aqueous phases were less than the detection limit.

Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date last updated: December 3, 2003.

Water Solubility (CAS No. 11138-60-6)

Test Substance	Decanoic acid, ester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol octanoate
CAS Number	11138-60-6
Remarks	Purity not indicated
Method/guideline	OECD 105
Test type	Water solubility
GLP	Not indicated
Year	1996
Procedure	The flask method of OECD 105 was used. The water solubility was determined in ultrapure water. 4 mL (~3.8 g) test material was added to 47 mL of solvent in a 50 mL vial (duplicate samples). The vials were shaken at 22±1°C for 2.1 and 4.8 days. Following centrifugation, the water samples were sampled with a syringe, extracted with 2 mL of methyl *t*-butyl ether and the organic extracts were analyzed by GC-FID.
Results /Remarks	Concentration in test solutions after 2.1 and 4.8 days was respectively 0.44 and 0.51 mg/L Water solubility of test material was determined to be 0.48 ± 0.14 mg/L at 22 ± 1°C Remarks/comments: 1) Test material's purity was not specified. 2) The pH during the test was not reported. Whether test material is significantly hydrolyzed under test conditions is unclear. 3) The limit of GC-FID detection (statistical estimate of the minimum concentration of test material in water that could be detected with 90% confidence) was 0.5 µg/mL. The result of the report was close to this value.
Conclusions	Water solubility of test material was 0.48 ± 0.14 mg/L at 22 ± 1°C.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP and reasons discussed above.
References	Unpublished confidential business information.
Other	Date last updated: December 5, 2003.

Acute Oral Toxicity (CAS No. 11138-60-6)

Test Substance	Decanoic acid, ester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol octanoate
CAS Number	11138-60-6
Remarks	Purity not indicated
Method/guideline	OECD 401
Test type	Acute oral toxicity
GLP	Yes
Year	1997
Test system	Species (Strain): Rats (Sprague-Dawley)

	Sex: Male and female; weight: 302-306 g (males), 208-216 g (females) No. of animals: 5 /sex/treatment Route: Single oral gavage Dosage: 5000 mg/kg (dosing volume 5.3 ml/kg, undiluted) Statist. Methods: None required
Test conditions	Five male and 5 female Sprague-Dawley rats were fasted for 18 hrs and dosed by gavage with 5000 mg/kg body weight of the test material. No controls; feeding *ad libitum* at ~4 hr after dosing. Observations for mortality were carried out twice daily until day 15. Clinical signs of toxicity were observed three times on day 1 and daily until day 15. Body weights were measured on day 0, 1, 8 and 15. Necropsy was performed on day 15.
Results/Remarks	No mortality or clinical signs of toxicity were observed in any of the female or male rats. There were no treatment-related body weight changes. No abnormalities or gross lesions were observed at necropsy.
Conclusions	The oral LD50 was > 5000 mg/kg.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date last updated: December 5, 2003.

Repeated-Dose Toxicity (CAS No. 11138-60-6)

Test Substance	Decanoic acid, ester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol octanoate
CAS Number	11138-60-6
Remarks	Purity not indicated
Method/guideline	Not indicated but meets test procedures required by OECD 410
Test type	28-Day Dermal toxicity study in rats
GLP	Not indicated
Year	1997
Species/strain	Rats /Sprague-Dawley, age 7 weeks, weight 147-220 g (males), 140-177 g (females)
Route of Administ.	Dermal administration of test material on clipped dorsal skin (no vehicle)
Duration of test	28 days. Additional 14 days for recovery groups in control and high dose animals.
No. of animals	10/sex/dose level; additionally 10/sex in control and high dose group for 14-day recovery
Dose/Conc. Levels	0 (untreated control), 125, 500 and 2000 mg/kg/day
Sex	Male and female
Frequency of treatment	Daily dermal administration, 5 days/week for 4 weeks (28 days)
Control Group	Yes. Untreated control group. Also group of 10 control rats for 14-day recovery after 28 day dermal administration
Post-exposure observat.	Mortality, survival, growth, food consumption, clinical signs/symptoms, clinical chemistry, hematology, necropsy, gross morphology and histopathology appear to have been carried out.
Statist. Methods	ANOVA, Dunnett's test
Post-exposure observat.	Mainly those required by OECD 410 test guidelines.
Results	

Dose mg/kg bw)	**0**		**125**		**500**		**2000**		**2000** (recovery)		**Dose-related**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mortality	None											
Clinical signs [(a)]	+	+	+	+	+	+	+	+	+	+		

Local effects [(b)]			+	+	+	+	+	+	+	+		
Body weight			dc				dc	dc	dc	dc		
Body weight gain							dc	dc				
Food consumption (day 0-7)							dc					
Haematology												
Lymphocytes				dc			dc					
Neutrophils				ic			ic	ic				
MHCH								dc				
RBC									dc	dc		
MCV									dc			
Hb										dc		
Clinical chemistry												
Glucose							dc					
Creatinine					dc		dc	dc				
Albumin							dc	dc				
Albumin/globulin							dc		dc			
ALAT								ic		ic		
BUN				ic				ic				
Total bilirubin								dc				
Organ weight												
Kidney				ic^r		ic^r		ic^r				
Liver								ic^r				
Heart								ic^r				
Brain							ic^r	ic^r				
Testes							ic^r					
Thymus								dc^a				
Necropsy	No treatment related effects											
Histopathology [(c)]	No treatment related effects											

Abbreviations:
ic = increase (significant) i = increase dc= decrease (significant) d = decrease
r= relative to body weight x = dose-related + = effect present

Footnotes:
(a) Symptoms included poor grooming, (red) staining around eyes and nose, scab formation (neck), sparse hair coat and hair loss. These effects probably attributed to the wearing of collars to prevent animals from grooming and orally ingesting of the test substance on skin.
(b) Effects included erythema, skin sloughing and paleness of the skin (no local effects during the first week of the study).
(c) Hypotrichosis, epidermal hyperplasia, epidermatitis, hyperkeratosis, edema, ulceration, abscesses and foreign body granuloma were seen in the skin and subcutis of the neck region (related to the collars animals wore).

Conclusions

NOAEL was 2000 mg/kg b.w. based on no evidence of microscopic changes in histopathological examination.

Remarks/comments

1) The effects noted as a result of treatment (viz, decrease in body weight and serum protein values) were slight and of slight and of little toxicological concern.
2) The effects on organ weights can be related most probably to the lower body weights observed in high dosed animals. For relative kidney weight the effect was related to a slight, not significant reduction of body weight at 125 and 500 mg/kg in females.
3) The effects on the number of lymphocytes were coincidental, since they were not seen in the opposite sex. A decreased creatinine level is toxicological irrelevant.
In male recovery animals (2000 mg/kg bw) additionally increased levels of sodium, potassium, phosphate and triglycerides were seen.
4) The application area was not indicated and may have been larger than 10% of the total body surface area. Since animals wore collars to prevent oral ingestion of the test substance, the test site was left uncovered (OECD 410 indicated a porous dressing to be applied), which may influence absorption

Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP.
References	Unpublished confidential business information.
Other	Date last updated: December 5, 2003.

Genetic Toxicity In Vitro (CAS No. 11138-60-6)

Test Substance	Decanoic acid, ester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol octanoate
CAS Number	11138-60-6
Remarks	Purity not indicated
Method/guideline	Not indicated but procedures comply with OECD 471 guidelines
Type of Study	Bacterial Reverse Mutation Assay
Test System	Bacterial (*Salmonella - Escherichia coli*)
GLP	Yes
Year	1996
Species/Strain	*Salmonella typhimurium* / TA1535, TA1537, TA1538, TA98, TA100 and *Escherichia coli* / WP2uvrA
Metab. Activation	Aroclor 1254-induced rat liver preparations (S9 mixture)
Concentrations	10, 33, 100, 333, and 1000 µg/plate of the test material (without S9 mix) 33, 100, 333, 1000 and 5000 µg/plate of the test material (with S9 mix)
Statist. Methods	Not specified but positive controls were run concurrently with test substance.
Test Conditions/ Remarks	Ethanol was used a vehicle (negative) control. Concurrent positive control materials were 2-aminoanthracene (all strains with S9); 2-nitrofluorene (TA98, TA1538), sodium azide (TA100, TA1535), 9-aminoacridine (TA1537), methyl methanesulfonate (*E. coli* WP2 uvrA) (all without S9)
Results	The test substance was negative for mutagenic activity in the five *Salmonella* tester strains and in the *E. coli* strain, with or without metabolic activation. No mutagenic activity was observed at concentrations ranging from 10µg/plate to the highest concentration of 5000 µg/plate. The bacterial strains tested included *Salmonella typhimurium* strains TA1535, TA1537, TA 1538, TA98; TA100 and *Escherichia coli* strain WP2uvrA. The negative (vehicle) control and positive controls gave the appropriate responses as expected. Precipitate was observed at ≥100 to 5000 µg/plate. No appreciable toxicity was observed.
Conclusions	The test substance was <u>not</u> mutagenic, with or without metabolic activation in the *Salmonella-Escherichia coli* / Mammalian Microsome Reverse Mutation assay.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date last updated: December 5, 2003.

Genetic Toxicity In Vitro (CAS No. 11138-60-6)

Test Substance	Decanoic acid, ester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol octanoate
CAS Number	11138-60-6
Remarks	Purity not indicated
Method/guideline	Not indicated
Type of Study	In Vitro Mammalian Chromosomal Aberration Test

Test System	Chinese hamster ovary (CHO) cell line
GLP	Yes
Year	1996
Species/ cell type	CHO cells
Metab. activation	Arochlor 1254-induced rat liver S9 mixture
Concentrations	625, 1250, 2500 and 5000 µg/ml (based on limited toxicity) Negative vehicle control was ethanol
Statist. Methods	Fisher's exact test, Cochran-Armitage test
Test Conditions /Remarks	Study was carried out to assess the ability of test substance to induce chromosomal aberrations in CHO cells cultured in vitro. Solvent and positive control cultures were also prepared. Two hours before the end of the incubation period, cell division was arrested with Colemid, the cells harvested and slides prepared so that the metaphase cells could be examined for chromosomal damage. Negative Control: vehicle (ethanol) Positive Controls: mitomycin-C (-S9), cyclophosphamide (+S9) On the basis of these data, the following concentrations were selected for metaphase analysis: <u>625, 1250, 2500 and 5000 µg/ml dose levels</u> 1) without S9: 4 h exposure + 16 h recovery. 2) without S9: 20 h exposure. 3) with S9: 4 h exposure + 16 h recovery. Colcemid was added for the last 2 hours.

Results

Exposure (h)	Metabolic activation	Doses tested [µg/ml]	Aberrations [%] at doses, respectively	Test result
4	Without	625, 1250, 2500, 5000	0, 2, 2, 0	Negative
	With	625, 1250, 2500, 5000	2, 3.5, 2, 1	Negative
20	Without	625, 1250, 2500, 5000	1, 2, 2.5, 1.5	Negative

Remark/comment	1) The positive and vehicle solvent negative controls gave the expected responses to fulfill the requirements of a valid test. 2) Experiment without metabolic activation was performed twice, but only the results of the second test were presented in report.
Conclusions	The test material is <u>not</u> clastogenic in the CHO cell culture test system, with or without metabolic activation. Regardless of dose level (from 625 µg/ml to as high as 5000 µg/ml) and dosing regimen, the test substance was concluded to be negative for structural and numerical chromosome aberrations, with or without S-9.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date last updated: December 5, 2003.

Developmental Toxicity (CAS No. 11138-60-6)

Test Substance	Decanoic acid, ester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol octanoate
CAS Number	11138-60-6
Remarks	Purity - Nominal level of 100%
Method/guideline	OECD and U.S. FDA Guidelines
Test type	Developmental toxicity study
GLP	This study was conducted in compliance with GLP regulations of the OECD, MHW and U.S. FDA
Year	1997
Species/strain	Rat/ Sprague-Dawley-Crl:CD® BR VAF/Plus®, 12 weeks old and 225 g mean b.w.
Route of Administ.	Dermal
Duration of test	Gestation days (GD) 6-15
Sex, No. of animals	Female (pregnant), 25 / dosage group
Dose/Conc. Levels	200, 600 and 2000 mg/kg body weight in corn oil
Frequency of treatment	Daily on each gestation day 6-15 (duration of 6 hrs per day)
Control Group	0 mg/kg, vehicle corn oil control only
Statist. Methods	Data were analyzed using analysis of variance (ANOVA) (Snedocor and Cochran, 1967); Dunnett's test (Dunnett, 1955); Bartlett's test; Kruskal-Wallis test (Sokal and Rohlf, 1969); Fisher's exact test (Siegel, 1969)
Remarks on Test Conditions	Mated female rats were dosed dermally on gestation day 6 up to day 15 post coitum (pc). Observations: mortality and clinical signs of dams were noted daily from day 0 to 20. Body weight was recorded on day 0, 6, 16 and 20. Body weight gains were calculated based on body wt on day 0 of gestation. All females were sacrificed and subjected to macroscopic examination of all maternal organs on day 20. The uteri were removed, weighed and examined for number of corpora lutea, number of implantation sites and number and location of fetuses and resorptions. Fetuses were inspected on total number, sex, weight, external and visceral defects (½ of fetuses by the modified Wilson technique and ½ of fetuses were cleaned in potassium hydroxide and stained with Alizarin red by Dawson's technique). Visceral examination was performed and alterations of fetuses classified into four categories: variations, retardations, anomalies and malformations
Results	Maternal data: Dermal application of 600 and 2000 mg/kg/day dosages of the test article caused local irritation (erythema, flaking, edema, and scabbing). The NOAEL of 200 mg/kg for maternal toxicity. Developmental data: The developmental NOAEL is greater than 2000 mg/kg/day (no adverse effects on embryo-fetal number, viability, sex ratio, body weight or morphology were observed at the highest dosage tested). As evaluated for the embryo/fetotoxicity and teratogenicity, the NOAEL was >2000 mg/kg b.w.
Conclusions	As evaluated in this study, dermal application of this test article at a dosage of 2000 mg/kg/day was not selectively toxic to female reproductive performance or development of the offspring.
Data Quality	Reliable without restrictions [Klimisch reliability 1]

References	Unpublished confidential business information supplied to the ACC Aliphatic Esters Panel.
Other	Date: June 11, 2004.

Acute fish toxicity (CAS No. 11138-60-6)

Test Substance	Decanoic acid, ester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol octanoate
CAS Number	11138-60-6
Remarks	Purity was 100%
Method/guideline	OECD 203; EC L 383A/163-171 C 1 (1992)
Type (test type)	Acute fish toxicity study
Test System	Fish, freshwater
GLP	Yes
Year	1996
Species/Strain	Fish: Rainbow trout (*Oncorhynchus mykiss)*
Analyt. Monitoring	Analyses were performed by GC-FID
Exposure period	96 hours
Statist. Methods	Binomial probability analysis (Stephan *et al.*, 1978)
Test Conditions	96-hr static acute fish toxicity test at five nominal concentrations from 65 mg/L to 1035 mg/L Species: Rainbow trout (*Oncorhynchus mykiss*), mean length 30-32 mm Test performed in 40 L glass vessels containing 30 L well water (hardness 203 mg/L $CaCO_3$); 12±1°C; 16 h light/8h dark cycle; unfed; loading 0.2-0.3 g/L. The test substance (oil) was maintained as oil in water dispersion/suspension by a propeller (protected against the fish) above the system, which created a vortex of 0.6-1.3 cm. No. of fish: 20/treatment Concentrations (nominal): 0 (untreated controls), 65, 129, 259, 517 and 1035 mg/L Physical Measurement: The pH, temperature and dissolved oxygen were performed daily. During course of 96 hr study, the pH ranged from 7.8 to 8.2, dissolved oxygen was 77-90% of saturation, and temperature was 11-13°C. Observations: Mortality/symptoms at 24, 48, 72 and 96 hr GC limit of detection for test material was 0.12 mg/L.
Result	Nominal test conc. Loading Level (mg/L) — Mortality (96-hr) 0 Control (untreated) — 0 65 — 0 129 — 0 259 — 0 517 — 0 1035 — 5 No mortality was observed in the fish at nominal concentrations from 65 mg/L to 517 mg/L and only about 5% (1/20 fish) were affected at the highest concentration of 1035 mg/L.
Conclusion	The 96-h LC_{50} was >1035 mg/L (nominal concentration, oil in water suspension/dispersion). Nominal test concentrations were all above the water solubility of the test material (experimentally determined to be 0.48 mg/L). GC-FID analysis revealed that test material was present in water samples and this is not unexpected since test material was mechanically dispersed in water. Hence, ecotoxicity data indicate that the test material would not be expected to cause acute toxicity in fish at its water saturation limit or water solubility limit (WSL).
Remarks	1) The fish were relatively small (30 mm, EC L 383 A: 60±20 mm). Since small fish may be more sensitive, this may be acceptable in a worst case approach. 2) Because the test substance is not soluble in water, it is kept in suspension by a propeller situated above the water surface, utilizing oil in water dispersion method.

	3) The LC50 is determined using the nominal concentration, since test material was water-insoluble. 4) The temperature during the study was at the lower range of temperature recommended (11-13°C versus EC L 383 A recommended 12-17°C).
Data Quality	Reliable with restrictions [Klimisch reliability 2].
References	Unpublished confidential business information.
Other	Date last updated: December 8, 2003.

Acute fish toxicity (CAS No. 11138-60-6)

Test Substance	Decanoic acid, ester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol octanoate
CAS Number	11138-60-6
Remarks	Purity was 100%
Method/guideline	EEC L 251/146-154, C1
Type (test type)	Acute fish toxicity study
Test System	Fish, saltwater
GLP	No
Year	1991
Species/Strain	Fish: Sheepshead minnow (*Cyprinodon variesatus*)
Analyt. Monitoring	No analyses were performed
Exposure period	96 hours
Statist. Methods	Binomial probability analysis (Stephan *et al.*, 1978)
Test Conditions	96-hr static acute fish toxicity test at five nominal concentrations from 101 to 5045 mg/L Species: Sheepshead minnow (*Cyprinodon variesatus*), weight 0.08-0.1 g Test performed in 40 L glass vessels containing 30 L of synthetic seawater (salinity 20±2 ppt) at 20±2°C, 16 h light/8 hr dark, unfed. The test substance (oil) was maintained as oil in water dispersion/suspension by a propeller (protected against the fish) above the system which created a vortex of 0.6-1.3 cm. No. of fish: 20/treatment Concentrations (nominal): 0 (untreated controls), 101, 504, 1009, 2018 and 5045 mg/L Physical Measurement: The pH, temperature and dissolved oxygen were performed daily. During course of 96 hr study, the pH ranged from 8.1 to 8.4, dissolved oxygen was 81-101% of saturation, and temperature was 21-22°C. Salinity was 20-21 ppt. Observations: Mortality at 96 hr
Result	Nominal test conc. Loading Level (mg/L) — Mortality (96-hr) 0 Control (untreated) — 0 101 — 0 504 — 0 1009 — 5 2018 — 0 5045 — 5
Conclusion	The 96-h LC_{50} was > 5045 mg/L (nominal concentration, oil in water suspension/dispersion). Nominal test concentrations were all above the water solubility of the test material (experimentally determined to be 0.48 mg/L). Hence, ecotoxicity data indicate that the test material would not be expected to cause acute toxicity in fish at its water saturation limit or water solubility limit (WSL).

Remarks	1) Due to cloudiness of the test solutions mortality counts could only be performed at the end of the test for the three highest concentrations. Food was withheld only 24 h before start of the study. (OPPTS 850.1075, 48 h). Fish that are withheld from food are more sensitive 2) Because the test substance is not soluble in water, it is kept in suspension by a propeller situated above the water surface, utilizing oil in water dispersion method. 3) The LC50 is determined using the nominal concentration, since test material was water-insoluble.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP and no chemical analysis were carried out on the tested water solutions.
References	Unpublished confidential business information.
Other	Date last updated: December 8, 2003.

Acute toxicity to aquatic invertebrate (CAS No. 11138-60-6)

Test Substance	Decanoic acid, ester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol octanoate
CAS Number	11138-60-6
Remarks	Purity was 100%
Method/guideline	OECD 202, EEC Directive 92/69/EEC L383 A
Type (test type)	*Daphnia sp.* , Acute immobilization test
Test System	Freshwater invertebrate
GLP	Yes
Year	1996
Species/Strain	Freshwater invertebrate, *Daphnia magna*
Analyt. Monitoring	Analyses were performed by GC-FID of samples collected at 0 and 48 h for WAF concentrations of 0, 24, 242 and 2570 mg/L
Exposure period	48 hours
Statist. Methods	Binomial probability analysis (Stephan *et al.*, 1978)
Remarks on Test Conditions	48-hr static immobilization study Species *Daphnia magna*, <24 h old Test was performed at 20°C in 250 mL glass beakers containing 200 mL water of hardness 203 mg/L ($CaCO_3$), 16 hr light/8 hr dark cycle, unfed No. of daphnids: 10 /replicate, 2 replicates/treatment Concentrations (nominal): 0 (untreated controls), 24, 97, 242, 1018 and 2570 mg/L as water accommodated fractions (WAF). Physical measurements: At 0 and 48 hr in all concentrations, pH, dissolved oxygen and temperature were performed; range for pH was 8.1-8.5; dissolved O_2 was 89-95% of saturation; temperature was maintained at 20°C. Observations: Immobility and symptoms at 0, 24 and 48 hr Chemical analyses of test material were carried out by solvent extraction from collected water samples and quantitated by GC/FID. GC limit of detection of test material was 0.12 mg/L.
Results	WAF Solution Conc. Nominal load rate (mg/L) — Immobility % (48-hr) 0 Control (untreated) — 5% 24 — 0 97 — 0 242 — 0 1018 — 0 2570 — 0

	GC-FID analysis for water samples at 0 hr indicated that test material was present at measured concentrations ranging from 0.13 to 0.41 mg/L. This is close to or lower than the water solubility of the test material.(experimentally determined as 0.48 mg/L.
Conclusions	48-hr EC_{50} was > 2570 mg/L WAF (nominal loading rate). No immobilization or adverse symptom was observed in the daphnids at any of the tested WAFs. Test material was shown to be present in WAF solutions and measured levels were close to the water solubility limit or water-saturated levels (WSL) of the test material. The data would suggest that test substance did not cause immobilization at or close to its water saturation levels or water solubility limits (WSL).
Remarks	WAF is the maximum soluble concentration of the nominal test concentrations after 20 hours of stirring and 4 hours of equilibrating. Only the water phase was used in the definitive test solutions.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Chemical analyses were based on limited number of measured samples.
References	Unpublished confidential business information.
Other	Date last updated: December 8, 2003.

Acute toxicity to aquatic plants (e.g., algae) (CAS No. 11138-60-6)

Test Substance	Decanoic acid, ester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol octanoate
CAS Number	11138-60-6
Remarks	Purity was 100%
Method/guideline	OECD 201, EEC L383A/179-186 C3 (1992)
Type (test type)	Algae, growth inhibition test
Test System	Aquatic plant (e.g., algae)
GLP	Yes
Year	1996
Species/Strain	Green algae / *Raphidocelis subcapitata* (formerly, *Selenastrum capricornutum*)
Analyt. Monitoring	Analyses were performed
Exposure period	72 hours
Statist. Methods	Fischer's exact test and binomial probability analysis
Test Conditions/ Remarks	Static 72 hr algae growth inhibition study Species: Green algae (*Raphidocelis subcapitata*, formerly, *Selenastrum capricornutum*) Tests were performed in 125 mL flasks containing 50 mL of algal medium (pH 7.5); temperature: 24±1°C; continuous illumination (~5000 lux); continuously shaken at 100 rpm Initial Cell Conc.: 1 x 10^4 cells/mL No. of replicates: 3 per treatment, 6 for controls Concentrations (nominal): 0 (untreated controls), 12, 24, 97, 242 and 1018 mg/L, water accommodated fractions (WAF) prepared at nominal loading rates Physical Measurements: The pH and temperature were performed. The range of pH was 7.8 to 8.4 at 72 hr in all flasks; temperature maintained at 24±1°C. Observations: Cell density at 24, 48 and 72 hr by counting with hemacytometer Chemical analyses of test material were carried out by solvent extraction from collected water samples and quantitated by GC/FID. GC limit of detection of test material was 0.12 mg/L.

Results							
		WAF Solutions (nominal loading rates) [mg/L]					
Parameter	Time [hr]	0	12	24	97	242	1018
Mean cell density [10^4 cells/ml]	24	8	8	7	7	7	4
	48	29	22	26	24	25	13
	72	129	106	106	114	122	82
% Inhibition - AUC	0-72	0	18	15	13	8	44
% Inhibition - growth rate	0-72	0	4	4	3	1	9

Remark/comment	1) WAF is the maximum soluble concentration of the nominal test concentrations after 20 hours of stirring and 4 hours of equilibrating. Only the water phase was taken and tested. 2) The analytical results show very low concentrations of the test material in WAF solutions. GC-FID analysis for water samples at 0 hr indicated that test material was present at measured concentrations ranging from 0.54 to 1.24 mg/L. This is close to or slightly above the water solubility of the test material (experimentally determined as 0.48 mg/L). 3) Light intensity and algae medium were not in accordance with OECD 201. The test is still acceptable, since no effects on the cell growth was seen in the controls.
Conclusions	72-hr EC_{50} was estimated to be > 1018 mg/L WAF (nominal loading rate) Test material was shown to be present in WAF solutions and measured levels were close to or slightly above the water solubility limit or water-saturated levels (WSL) of the test material. The data suggest that test material would not be expected to cause aquatic toxicity at or close to its water saturation levels or water solubility limits (WSL).
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Chemical analyses were based on limited number of measured samples.
References	Unpublished confidential business information.
Other	Date last updated: December 8, 2003.

Biodegradation (CAS No. 11138-60-6)

Test Substance	Decanoic acid, ester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol octanoate
CAS Number	11138-60-6
Remarks	Purity was 100%
Method/guideline	EPA 560/6-82-003 (equivalent to OECD 301B methodology) Shake Flask Aerobic Biodegradation - CO_2 evolution method using non-acclimated inoculum
Test type	Aerobic Biodegradation - CO_2 evolution method
GLP	No
Year	1993
Test system	Exposure Period: 28 Days Inoculum: Activated Sludge, Domestic, Unacclimated. Kinetics: Not Reported Biodegradation Products: Not Reported Analytical Monitoring: CO_2 evolution monitored in traps containing base solution.
Test Conditions	Inoculum: Activated sludge obtained from wastewater treatment plant. Amount inoculum added was sufficient to final inoculum solids conc. of 30 mg solids/L. Duplicate flasks Treated [medium + inoculum + test material (10 mg C/l)]; Duplicate flasks Treated [medium + inoculum + test material (20 mg C/l)]; Duplicate flasks Positive Control [medium + inoculum + sodium benzoate (20 mg C/l))]; Duplicate Blank Control [medium + inoculum].

	Incubation was performed under continuous shaking in 2L flasks, containing 1L of medium, test substance and/or inoculum at 25±3 ^{0}C in the dark. Evolved CO_2 was collected in appropriate trap containing 10 ml 0.2N KOH. CO_2 was monitored at various time points over a period of 28 days. Flask CO_2 traps were sampled at days 2, 5, 9, 14, 22 and 28. The amount of CO_2 was determined in the traps by back titration with 0.2N HCl, after addition of $Ba(Cl)_2$ and indicator. One day prior to the final sampling, the medium was acidified with 1 ml of concentrated sulfuric acid. Blank controls were used to subtract for background CO_2 production. Concentrations for Test Substance was 10 mg C /L and 20 mg C/L for test substance. Concentration for sodium benzoate (positive control) was 20 mg C/L.
Results	**Biodegradation Results:** % Biodegradation [% of ThCO2] mean value

Day	2	5	9	14	21	28
Test Material (10 mg C/L)	5.0	34	53	58	64	67
Test Material (20 mg C/L)	6.7	38	54	58	62	64
Positive Control (sodium benzoate 20 mg C/L)	44	77	83	84	87	90

	Test material did not meet "10-day window" criteria for ready biodegradability. Positive controls achieved 90% biodegradation in 28 days and met the "readily biodegradable" criteria.
Conclusions	Biodegradation was 64-67% in 28 days. The test substance was not readily biodegradable.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP. Test method used was essentially equivalent to OECD 301B test method. Temperature was carried out at ambient temperature.
References	Unpublished confidential business information
Other	Date last updated: December 8, 2003

Biodegradation (CAS No. 11138-60-6)

Test Substance	Decanoic acid, ester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol octanoate
CAS Number	11138-60-6
Remarks	Purity was 100%
Method/guideline	OECD 301B Modified Sturm, 92/69/EEC L383, C4
Test type	Aerobic Ready Biodegradability test (Modified Sturm - CO_2 evolution method)
GLP	Yes
Year	1996
Test system	Exposure Period: 28 Days Inoculum: Activated sludge from municipal sewage treatment plant Kinetics: Not Reported
Test Conditions	Inoculum: activated sludge from domestic wastewater treatment plant. Sufficient inoculum (7 ml) to provide final 30 mg suspended solids/L medium. Blank control [medium + inoculum] (n=2) Positive control [medium + inoculum + sodium benzoate (20 mg C/L)] (n=2 Treated [medium + inoculum + test material (20 mg C/L)]. (n=2) Medium was buffered mineral medium solution (initial pH taken) as outline in OECD 301B guidelines.

	Biodegradation experiments were performed in the dark under continuous stirring in 4 L glass vessels. The inoculum and medium (3 L) were pre-acclimated during 24 hours, and subsequently treated and aerated for 28 days at 23-24°C with CO_2-free air. The outcoming air was passed through 3 consecutive CO_2-traps containing 100 ml 0.0126 M $Ba(OH)_2$. The amount of CO_2 was determined in the traps by back-titrating with standardized 0.05M HCl at various time intervals (duplicate determinations). The pH was measured on day 28 in the individual vessels. Concentrations for Test Substance was 20 mg C /L for test substance. Concentration for sodium benzoate (positive control) was 20 mg C/L.
Results	Biodegradation occurred to the extent of 76% in 28 days for the test substance. The test substance did not meet the "10-day window" criterion for "readily biodegradable". Positive controls (sodium benzoate) achieved 78% biodegradation in 28 days and met the readily biodegradable classification. Biodegradation values were corrected for background CO_2 with blank controls. **Biodegradation Results:**

	% Biodegradation [% of ThCO2]						
Day	**1**	**3**	**6**	**10**	**14**	**21**	**28**
Test Substance	0.0	11	29	45	54	61	76
Positive Control (sodium benzoate)	5.9	26	46	65	71	75	78

Conclusions	The test substance was biodegraded to the extent of 76% in 28 days. Test material was not readily biodegradable.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information
Other	Date last updated: December 8, 2003

Melting Point (Pour Point) (CAS No. 126-57-8)

Test Substance	Nonanoic acid, triester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol
CAS Number	126-57-8
Remarks	Purity was 100%
Method/guideline	OECD 102
Test type	Melting point (pour point determined since material is liquid at room temp.)
GLP	Yes but no signed GLP statement in report.
Year	1997
Procedure	About 4 mL (~4 g) of the test material was placed in a 15 mL glass test tube. The tube was cooled in liquid nitrogen. The tube with the frozen content was removed and allowed to warm in the air. Every 15 seconds the temperature was measured in the test material (8 mm from bottom, center) to determine the pour point/melting point. The test (cooling, warming) was repeated three times, now with the sample in horizontal position during warming to allow observation of substance flow. The apparatus was calibrated with tap water. The pour temperature of water was 4°C according to the test.
Results /Remarks	Pour point was determined to be -53, -62 and -68 °C. Mean value = -61±8°C

	1) The method used in the test may have some uncertainty. Calibration of method with tap water was slightly lower than expected. The slightly lower value (-4°C instead of 0°C for distilled water) could be partly due to impurities in the tap water but may be related to uncertainty in test method. The study reliability may be slightly lower than anticipated. 2) Although it was stated that all laboratory work undertaken was done using Good Laboratory Procedures, no signed GLP statement was included in the report.
Conclusions	Melting Point (Pour Point) was determined to be -61 °C.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Signed GLP statement not given in report.
References	Unpublished confidential business information.
Other	Date last updated: December 8, 2003.

Boiling Point (CAS No. 126-57-8)

Test Substance **CAS Number** **Remarks**	Nonanoic acid, triester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol 126-57-8 Purity was 100%
Method/guideline	OECD 103
Test type **GLP** **Year**	Boiling point (modified of Siwoloboff's method) Yes 1997
Procedure	The test substance (40 mm) was put in a sealed glass Pasteur pipette and inserted into the injection port of a gas chromatograph (T_{max} 314±5°C) at 102±1 kPa
Results /Remarks	No condensation of a significant amount of test substance (temperature <314°C) and no significant bubbles were formed (314°C).
Conclusions	Boiling point >300°C at 102±1 kPa
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not clear if modification of original of Siwoloboff method will have significant impact on accuracy since bp was greater than 300 °C and was not carried out at temperature above that temperature. GLP statement is signed by the study director. Although an external GLP auditor was mentioned, this person did not sign the GLP statement.
References	Unpublished confidential business information.
Other	Date last updated: December 3, 2003.

Vapor Pressure (CAS No. 126-57-8)

Test Substance **CAS Number** **Remarks**	Nonanoic acid, triester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol 126-57-8 Purity was 100%
Method/guideline	OECD 104, ASTM D2879-92

Test type	Vapor pressure
GLP	No
Year	1997
Procedure	The isoteniscope method described in OECD 104 was used.
Results /Remarks	Temp. [°C] Vapor Pressure [Pa] 25 21 30 27 40 40 50 57 The limit of determination (LOD) was 13 Pa.
Conclusions	Vapor pressure at 25°C was 21 Pa.
Remarks	The recommended range of vapor pressures using this method is 10^2-10^5 Pa according to OECD 104 test guidelines. The vapor pressure of test material lies below this level. The OECD 104 test method recommends that the repeatability be in the range of 5-10%; however, the limited information in this study was not sufficient to estimate repeatability. Since all measured vapor pressures in this study were <100 Pa, the study reliability was considered lower due to repeatability uncertainties.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Vapor pressure value at 25°C was close to limit of determination and repeatability uncertainties.
References	Unpublished confidential business information.
Other	Date last updated: December 12, 2003.

Partition Coefficient (CAS No. 126-57-8)

Test Substance	Nonanoic acid, triester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol
CAS Number	126-57-8
Remarks	Purity was 100%
Method/guideline	Not indicated but partition coefficient based on water solubility determination (OECD 105)
Test type	Partition coefficient
GLP	No
Year	1997
Procedure	Based on water solubility results, it was assumed that the concentration of test material in the aqueous phase of a P_{ow} experiment could not be determined with acceptable accuracy. The P_{ow} test was not performed and P_{ow} was estimated based on the solubilities of test material in octanol and water. 1) n-Octanol and test material (0.1-10 g/mL) were placed in six 4 mL glass vials and mixed for ~1 hour at 23°C. 2) For all concentrations homogeneous (single phase) solutions were formed.
Results	Solubility in n-octanol and water were respectively >900 g/L and 8.4 mg/L (see robust summary for water solubility determination of test material). Log P_{ow} >2.8 at 23±1°C
Conclusions	Log P_{ow} >2.8 at 23 ± 1°C

Remarks/ Comments	1) Determination of solubility of test material in n-octanol was based on visual (subjective) evaluation. No analyses were performed. 2) This test can be used for the estimation of the log(P_{ow}) of test material. However, only the solubility of test material in n-octanol was determined in this test. The water solubility was determined in a separate study. The partition of a mixture of water and n-octanol may be estimated by using the separate solubilities. It is clear from this report that most of the test substance will be found in the octanol-phase. 3) Although it was stated that all laboratory work undertaken was done using Good Laboratory Procedures, no signed GLP statement was included in the report.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Signed GLP statement not given in report.
References	Unpublished confidential business information.
Other	Date last updated: December 9, 2003.

Water Solubility (CAS No. 126-57-8)

Test Substance	Nonanoic acid, triester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol
CAS Number	126-57-8
Remarks	Purity was 100%
Method/guideline	OECD 105
Test type	Water solubility
GLP	Yes
Year	1997
Procedure	The solubility in water was determined using the flask method of OECD 105. About 4 mL (~4 g) test substance was added to 45 mL ultrapure water in a 50 mL test tube. The test tubes were mixed on a rotary mixer (5 rpm) at 22-23°C [note 1] for 24, 70 and 139 hours. Following centrifugation and equilibration to room temperature (1 hour), TOC analysis (total carbon and total inorganic carbon content determined from calibration curves) was performed for water samples. A blank sample (ultrapure water) was also performed for 139 hrs and analyzed for background TOC.
Results /Remarks	Notes: 1) No information was reported on temperature control except that the air temperature was 22-23 °C. Therefore, it is assumed that this was temperature range for the study. 2) The pH during the test was not reported. The extent if any of hydrolysis products is unclear. Assumption is that the measured test material is the main component responsible for the TOC in samples analyzed. 3) GLP statement is signed by the study director. Although an external GLP auditor was mentioned, this person did not sign the GLP statement.
Conclusions	Water solubility of test material was 8.4 ± 0.1 mg/L at 22-23 °C after 139 hrs of mixing
Data Quality	Reliable with restrictions [Klimisch reliability 2]. See reasons discussed above.
References	Unpublished confidential business information.
Other	Date last updated: December 9, 2003.

Acute Oral Toxicity (CAS No. 126-57-8)

Test Substance	Nonanoic acid, triester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol
CAS Number	126-57-8
Remarks	Purity was 100%
Method/guideline	OECD 401
Test type	Acute oral toxicity
GLP	Yes
Year	1997
Test system	Species (Strain) Rats (Crl:CD), weight 238-261 g Sex: Male and female No. of animals: 5 /sex/treatment Route: Single oral gavage Dosage: 2000 mg/kg (undiluted), dosing volume 2.17 ml/kg b.w. Statist. Methods: Not applicable
Test conditions	Five male and 5 female Sprague-Dawley rats were fasted for ~17-20 prior to dosing. Single oral (gavage) of 2000 mg/kg bw (dosing volume 2.17 ml/kg bw) was administered; no controls; feeding *ad libitum* after dosing and throughout observation period. Observations: Mortality twice daily until day 13 and once on day 14. Clinical signs were observed several times on day 0 and daily until day 14. Body weights were measured on day 0, 7 and 14. Necropsy was performed on day 14
Results/Remarks	No mortality was observed in any of the female or male rats. There were no reports of any treatment-related effects on clinical signs of toxicity or body weight gain. There were no treatment-related effects, gross morphology or histopathology at necropsy.
Conclusions	The oral LD50 was > 2000 mg/kg.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date last updated: December 9, 2003.

Acute Oral Toxicity (CAS No. 126-57-8)

Test Substance	Nonanoic acid, triester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol
CAS Number	126-57-8
Remarks	Purity was 100%
Method/guideline	OECD 401, 67/548/EEC B1
Test type	Acute oral toxicity
GLP	Yes
Year	1988
Test system	Species (Strain) Rats (Wistar); weight: 284-298 g (males), 209-210 g (females) Sex: Male and female No. of animals: 5 /sex/treatment Route: Single oral gavage

	Dosage: 2000 mg/kg (undiluted), dosing volume 2.2 ml/kg b.w. Statist. Methods: Not applicable
Test conditions	Five male and 5 female Sprague-Dawley rats were fasted overnight prior to dosing. Single oral (gavage) of 2000 mg/kg bw (dosing volume 2.17 ml/kg bw) was administered; no controls; feeding *ad libitum* about 3 hrs after dosing and throughout observation period. Observations: Mortality and clinical signs were observed several times on the day of dosing (day 0) and once daily until day 14. Body weights were measured on day 0, 7 and 14. Necropsy was performed on day 14
Results/Remarks	No mortality was observed in any of the female or male rats. There were no reports of any treatment-related effects on clinical signs of toxicity or body weight gain. There were no treatment-related effects, gross morphology or histopathology at necropsy.
Conclusions	The oral LD50 was > 2000 mg/kg.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date last updated: December 9, 2003.

Acute Oral Toxicity (CAS No. 126-57-8)

Test Substance	Nonanoic acid, triester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol
CAS Number	126-57-8
Remarks	Purity was not indicated
Method/guideline	Not indicated
Test type	Acute oral toxicity
GLP	No
Year	1973
Test system	Species (Strain) Rats (Sherman-Wistar) Sex: Male and female No. of animals: 5 /sex/treatment Route: Single oral gavage Dosage: 5000 mg/kg (undiluted) Statist. Methods: Not applicable
Test conditions	Five male and 5 female Sprague-Dawley rats were fasted for ~24 hrs and dosed by gavage with 5000 mg/kg body weight of the test material. No controls. Observations for mortality/clinical signs of toxicity for 14 days.
Results/Remarks	No mortality was observed in any of the female or male rats. No report was made on clinical signs. No measurements on body weight and no necropsy were reported.
Conclusions	The oral LD50 was > 5000 mg/kg.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP and limited experimental information and findings in report.
References	Unpublished confidential business information.
Other	Date last updated: December 9, 2003.

Genetic Toxicity In Vitro (CAS No. 126-57-8)

Test Substance	Nonanoic acid, triester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol
CAS Number	126-57-8
Remarks	Purity was 100%
Method/guideline	Not indicated but procedures comply with OECD 471 guidelines
Type of Study	Bacterial Reverse Mutation Assay
Test System	Bacterial (*Salmonella - Escherichia coli*)
GLP	Yes
Year	1997
Species/Strain	*Salmonella typhimurium* / TA1535, TA1537, TA98, TA100 and *Escherichia coli* / WP2uvrA
Metab. Activation	Aroclor 1254-induced rat liver preparations (S9 mixture)
Concentrations	100, 333, 1000, 3330, and 5000 µg/plate of the test material
Statist. Methods	Not specified but positive controls were run concurrently with test substance.
Test Conditions/ Remarks	DMF was used a vehicle (negative) control. Concurrent positive control materials were 2-aminoanthracene (all strains with S9); sodium azide (TA100, TA1535), 2-nitrofluorene (TA98), ICR-191 (TA1537), 4-nitroquinoline-N-oxide (*E. coli* WP2 uvrA) (all without S9)
Results	The test substance was <u>negative</u> for mutagenic activity in the four *Salmonella* tester strains and in the *E. coli* strain, with or without metabolic activation. No mutagenic activity was observed at concentrations ranging from 100 µg/plate to the highest concentration of 5000 µg/plate. The bacterial strains tested included *Salmonella typhimurium* strains TA1535, TA1537, TA98; TA100 and *Escherichia coli* strain WP2uvrA. The negative (DMF vehicle) control and positive controls gave the appropriate responses as expected. Slight precipitate was observed at 333 µg/plate and above. This may indicate that test concentrations may be at solubility limit in DMF/water in test. However, this dose does not affect the validity of the test since there was not indication of any toxic effect seen.
Conclusions	The test substance was <u>not</u> mutagenic, with or without metabolic activation in the *Salmonella-Escherichia coli* / Mammalian Microsome Reverse Mutation assay.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date last updated: December 9, 2003.

Acute fish toxicity (CAS No. 126-57-8)

Test Substance	Nonanoic acid, triester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol
CAS Number	126-57-8
Remarks	Purity was 100%
Method/guideline	Niemitz, LTwS, Nr10, 1979
Type (test type)	Acute fish toxicity study
Test System	Fish, freshwater
GLP	Yes

Year	1988
Species/Strain **Analyt. Monitoring** **Exposure period** **Statist. Methods**	Fish: Carp (*Cyprinus carpio*) No analysis was performed 48 hours None
Test Conditions	48-hr static acute fish toxicity test at limited concentration Species: Carp (*Cyprinus carpio*), length 20-40 mm Test performed in 10 L glass vessels containing 5L medium (tap water) (pH 8.2, hardness 199 mg/L $CaCO_3$); aerated; unfed. The test substance was maintained and tested as suspension in water. No. of fish: 10/treatment Concentrations (nominal): 0 (untreated controls) and 1000 mg/L Physical Measurement: At 0 and 48 hr in control and in 1000 mg/L groups, the pH, temperature and dissolved oxygen were performed. During course of 48 hr study, the pH ranged from 8.0 to 8.3, dissolved oxygen was 83-94% of saturation, and temperature was 20-22°C. Observations: Mortality/symptoms at 1-5, 24 and 48 hr
Result	Nominal test conc. Loading Level (mg/L) — Mortality (48-hr) 0 Control (untreated) — 0 1000 mg/L — 0
Conclusion	The 48-hr LC_{50} was >1000 mg/L (nominal concentration, water suspension/dispersion). No mortality was observed in the fish at nominal concentrations 1000 mg/L Nominal test concentration was expected to be above the water solubility of the test material (experimentally determined to be 8.4 mg/L) since droplet . Test material was tested as suspension. Hence, the ecotoxicity data indicate that the test material would not be expected to cause acute toxicity in fish at its water saturation limit or water solubility limit (WSL).
Remarks	1) Limited experimental information in report. 2) Chemical analyses were not performed the only information about the homogeneity of the solution was the description of the test medium as a suspension of macroscopic droplets of test substance.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Limited experimental information and no analysis of test material in water samples.
References	Unpublished confidential business information.
Other	Date last updated: December 10, 2003.

Acute toxicity to aquatic invertebrate (CAS No. 126-57-8)

Test Substance **CAS Number** **Remarks**	Nonanoic acid, triester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol 126-57-8 Purity was 100%
Method/guideline **Type (test type)** **Test System** **GLP** **Year**	OECD 202, EEC Directive 92/69/EEC L383 A *Daphnia sp.* , Acute immobilization test Freshwater invertebrate Yes 1996

Species/Strain	Freshwater invertebrate, *Daphnia magna*
Analyt. Monitoring	Analyses were performed by GC-FID of samples collected at 0, 24 and 48 h for all WAF solutions
Exposure period	48 hours
Statist. Methods	None
Remarks on Test Conditions	48-hr static immobilization study Species *Daphnia magna*, <24 h old Test was performed at 18-20°C in 100 mL glass dishes (covered with mesh) in 2L dishes containing 2000 mL of medium with dispersed test material and having hardness ~240 mg/L ($CaCO_3$), 16 hr light/8 hr dark cycle, unfed No. of daphnids: 10 /replicate, 2 replicates/treatment Concentrations (nominal): 0 (untreated controls), 1.0, 2.4, 5.6, 13 and 32 mg/L as dispersions in water. Physical measurements: At 0 and 48 hr in all concentrations, pH, dissolved oxygen and temperature were performed; range for pH was 7.3-7.6; dissolved O_2 was 79-86% of saturation; temperature was maintained at 18-20°C. Observations: Immobility and symptoms at 24 and 48 hr Chemical analyses of test material were carried out by solvent extraction from collected water samples at all test concentrations and quantitated by GC/FID using internal standards.
Results	Water Solution Conc. / Measured Nominal load rate (mg/L) / Conc. Mean (mg/L) / Immobility % (48-hr) 0 Control (untreated) / 0.07 mg/L / 5% 1.0 / 0.4 / 0 2.4 / 0.9 / 0 5.6 / 1.8 / 5 13 / 3.8 / 0 32 / 9.3 / 15 GC-FID analysis for water samples (mean conc shown in table above) indicated that test material was present 30-43% of the nominal loading rates . The measured level of 9.3 mg/L at the highest nominal loading rate of 32 mg/L indicated that test material was close to its water solubility limit (experimentally reported to be 8.4 mg/L).
Conclusions	48-hr EC_{50} was > 9.3 mg/L (measured conc.) Test material was shown to be present in WAF solutions and measured levels were close to the water solubility limit or water-saturated levels (WSL) of the test material. The data would suggest that test substance would not be expected to cause significant immobility at or close to its water saturation levels or water solubility limits (WSL).
Data Quality	Reliable without restrictions [Klimisch reliability 1]. 48-hr EC50 value was based on measured concentrations and not nominal loading rates.
References	Unpublished confidential business information.
Other	Date last updated: December 10, 2003.

Acute toxicity to aquatic plants (e.g., algae) (CAS No. 126-57-8)

Test Substance	Nonanoic acid, triester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol
CAS Number	126-57-8
Remarks	Purity was 100%
Method/guideline	OECD 201, EEC L383A C3 (1992), ISO 8692:1989(E)
Type (test type)	Algae, growth inhibition test

Test System	Aquatic plant (e.g., algae)
GLP	Yes
Year	1996
Species/Strain	Green algae / *Scenedesmus subspicatus*
Analyt. Monitoring	Analyses were performed (GC-FID quantitation)
Exposure period	72 hours
Statist. Methods	Not specified
Test Conditions/ Remarks	Static 72 hr algae growth inhibition study Species: Green algae (*Scenedesmus subspicatus*) Tests were performed in containers with algal medium under continuous illumination and agitation (shaker). Initial Cell Conc.: 1 x 10^4 cells/mL No. of replicates: 3 per treatment, 6 for controls Concentrations: 0 (untreated controls), 0.1, 0.32, 1.0, 3.2 and 10 mg/L (as dispersions) Physical Measurements: The pH and temperature were performed. The range of pH was 7.2-9.5 at 0 and 72 hr in the test solutions and temperature was 21-23 °C. Observations: Cell density at 0, 24, 48 and 72 hr by particle counting and at 48 and 72 hr by spectrophotometer Chemical analyses of test material were carried out by solvent extraction from collected water samples (one replicate per treatment at 0, 24, 48 and 72 hr) and quantitated by GC/FID.

Results

		Nominal Concentrations of Dispersion Water Solutions Tested (mg/L)					
		0 (Control)	**0.10**	**0.32**	**1.0**	**3.2**	**10**
		Mean Measured Concentrations (mg/L)					
Parameter	**Time (hr)**	**0.1**	**0.14**	**0.16**	**0.32**	**1.0**	**4.4**
Mean cell density [10^4 cells/ml]	0	1	1	1	1	1	1
	48	11	8	8	8	6	2
	72	55	42	53	59	70	61
% Inhibition - AUC	0-72	0	26	10	5	-1	22
% Inhib.-growth rate	0-72	0	8	1	-1	-9	-2

Remark/comment	1) Chemical analysis had limitations below 0.3 mg/L due to the low limit of GC detection. 2) In the report no information is available about the light regime and intensity. Since no effect on the control cell growth was seen, the circumstances during the study can expected to be correct, or at least acceptable to create a valid test. 3) The result of the cell density at 24 hours was not reported. 4) Strong rises in pH were recorded. Such rises are often associated with strong cell growth, probably due to CO_2 depletion from test media.
Conclusions	72-hr EC_{50} was estimated to be > 4.4 mg/L (measured water concentration) Test material was shown to be present in water test solutions and measured levels were below or close to the water solubility limit or water-saturated levels (WSL) of the test material. The data suggest that test material would not be expected to cause aquatic toxicity below or close to its water saturation levels or water solubility limits (WSL).
Data Quality	Reliable without restrictions [Klimisch reliability 1]. 72-hr EC50 value was based on measured concentrations and not nominal loading rates.
References	Unpublished confidential business information.
Other	Date last updated: December 10, 2003.

Biodegradation (CAS No. 126-57-8)

Test Substance	Nonanoic acid, triester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol
CAS Number	126-57-8
Remarks	Purity was 100%
Method/guideline	OECD 301/B (1981), 84/449/EEC L251, C5 (1984)
Test type	Aerobic Ready Biodegradability: Modified Sturm - CO_2 evolution test method
GLP	Yes
Year	1991
Test system	Exposure Period: 28 Days Inoculum: Activated Sludge, Domestic, Unacclimated. Kinetics: Not Reported Biodegradation Products: Not Reported Analytical Monitoring: CO_2 evolution monitored in traps containing base solution.
Test Conditions	Inoculum: from activated sludge from a municipal sewage treatment plant. Amount inoculum 10 ml/l (1%) added to each flask. Treated [medium + inoculum + test material (10 mg /L = 7.1 mg C/L)]; Treated [medium + inoculum + test material (20 mg /L = 14.3 mg C/L)]; Positive Control [medium + inoculum + sodium acetate (20 mg/L = 5.9 mg C/L)]; Duplicate Blank Control [medium + inoculum]. Biodegradation experiment was performed under continuous stirring in brown 3 L glass flasks containing 3000 ml of mineral solution with test substance and/or inoculum. The inoculum was pre-acclimated for 24 h, treated and aerated for 28 days at 20±2°C with CO_2-free air. The outcoming air was passed through 3 consecutive CO_2-traps containing 0.025N $Ba(OH)_2$. The amount of evolved CO_2 was determined in the traps by back-titration of residual $Ba(OH)_2$ at various time intervals (2, 5, 7, 9, 12, 16, 21 and 28 days. Blank controls were used to correct for subtraction of background CO_2. Concentrations for Test Substance was 7.1 mg C /L and 14.3 mg C/L for test substance. Concentration for sodium acetate (positive control) was 5.9 mg C/L.
Results	**Biodegradation Results:**

% Biodegradation [% of ThCO2] mean value

Day	2	5	7	9	12	16	21	28
Test Material (7.1 mg C/L)	0	4.3	13	17	22	29	36	43
Test Material (14.3 mg C/L)	0	1.2	16	27	37	45	51	54
Positive Control (sodium acetate 5.9 mg C/L)	6.2	17	24	28	37	61	96	111*

* due to acidification.

Conclusions	Biodegradation was 43-54% in 28 days. The test substance was not readily biodegradable.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Composition nutrient solution not in accordance with OECD 301 B. No replicate flasks were included. Positive control was sodium acetate (allowable by guidelines) but other reference such sodium benzoate could have been used.
References	Unpublished confidential business information
Other	Date last updated: December 10, 2003

Acute Oral Toxicity (CAS No. 70024-57-6)

Test Substance	9-Octadecenoic acid (Z)-, ester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol
CAS Number	70024-57-6
Remarks	Purity not specified. Mixture containing CAS No. 70024-57-4 and CAS No. 67989-24-6 [9-Octadecenoic acid (Z)-, ester with 2,2-dimethyl-1, 3-propanediol] was tested; composition not specified.
Method/guideline	Not indicated
Test type	Acute oral toxicity
GLP	No
Year	1976
Test system	Species (Strain) Rats (strain not specified) Sex: Male rats, weight 205-237 g No. of animals: 5 males/dose group Route: Oral gavage Dosage: Single oral administration (gavage) of 0.464, 1.00, 2.15, 4.64 and 10.0 ml/kg bw; no controls; feeding *ad libitum* but food was withheld ~18 h prior to dosing. Statist. Methods: Not specified.
Test conditions	Test material was administered to groups of 5 male rats, fasted for 18 hrs at the six dose concentrations cited above. Observations included: (1) Mortality/clinical signs several times on day 1 and at least once daily for 14 days. (2) body weights on day 1 and 14; (3) necropsy on day 14.

Results

		Dosage Levels					
Endpoint or Effect , Observ.	**Day**	**0.464 ml/kg**	**1.00 ml/kg**	**2.15 ml/kg**	**4.64 ml/kg**	**10.0 ml/kg**	**Dose related Effect**
Mortality	1-14	None					
Clinical signs[(A)]	1-14			+	+	+	x
Body weight gain	1-14	No treatment related effects					
Necropsy	14	No treatment related effects					

Abbreviations/footnotes:
+ = Clinical observations reported were diarrhea, oily rough fur, depression, depressed righting and placement reflexes
x = does-related effect observed

Remarks	Other remarks: males/dose group was used instead of 5/sex/dose group. No measurements of body weight were performed on day 7.
Conclusions	The oral LD50 was > 10 ml/kg.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP. Limited report.
References	Unpublished confidential business information.
Other	Date last updated: December 10, 2003.

Acute fish toxicity (CAS No. 70024-57-6)

Test Substance	9-Octadecenoic acid (Z)-, ester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol
CAS Number	70024-57-6
Remarks	Purity not specified. Mixture containing CAS No. 70024-57-4 and CAS No. 67989-24-6 [9-Octadecenoic acid (Z)-, ester with 2,2-dimethyl-1,3-propanediol] was tested; composition not specified.
Method/guideline	OECD 203 (1981 guidelines)
Type (test type)	Acute fish toxicity study
Test System	Fish, freshwater
GLP	No
Year	1993
Species/Strain	Fish: Rainbow trout (*Oncorhynchus mykiss*)
Analyt. Monitoring	No analyses were performed.
Exposure period	96 hours
Statist. Methods	Trimmed Spearman Karber analysis
Test Conditions	96 hr static test acute fish toxicity study at five nominal test concentrations Species: Rainbow trout (*Oncorhynchus mykiss*), length ~50 mm Test was performed in 20 L glass vessels containing 6 L of water (hardness 66-68 mg/L $CaCO_3$); 15±1°C; 16 h light/8hr dark cycle; unfed; aerated. No. of fish: 10/vessel, 2 vessels/treatment Concentrations (nominal): 40.5, 135, 450, 1500 and 5000 ppm (v/v), untreated controls The test substance (oil) was emulsified using a blender Physical measurements: Daily in all vessels: overall ranges for pH 7.1-7.5; O_2 60-83%; temperature 14-16°C Observations: Mortality/symptoms at 24, 48, 72 and 96 h
Results	Nominal test conc. Loading Level (ppm, v/v) — Mortality (96-hr) 0 Control (untreated) — 0 40.5 — 0 135 — 0 450 — 5 1500 — 20 5000 — 100
Conclusion	The 96-h LC50 was estimated to be 2027 ppm (v/v) (equivalent to ~2000 mg/L if density of ~1.0 was assumed). The 96-h LC_0 was 135 ppm (nominal) in which no mortality was observed. Test concentrations were all above the water solubility of the test material (calculated to be 0.0010 mg/L, EpiWin). Hence, data indicate that the test material would not be expected to cause acute toxicity in fish at its water saturation limit or water solubility limit (WSL).
Remarks	1) The biological loading was not specified in the report. It is not clear if the biological loading exceeded 1 g fish/L, since a mean weight of 0.6 gram for fish with a length of ~50 mm appears to be rather low. 2) Because the test substance is not soluble in water, a suspension of the test substance in water is used. The emulsions were reported to be reasonable stable, but surface pooling was observed.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP and for reasons discussed above.
References	Unpublished confidential business information.

Other	Date last updated: December 10, 2003.

Biodegradation (CAS No. 70024-57-4)

Test Substance **CAS Number** **Remarks**	9-Octadecenoic acid (Z)-, ester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol 70024-57-6 Purity not specified. Mixture containing CAS No. 70024-57-4 and CAS No. 67989-24-6 [9-Octadecenoic acid (Z)-, ester with 2,2-dimethyl-1,3-propanediol] was tested; composition not specified.
Method/guideline **Test type** **GLP** **Year**	Modified Sturm Test, 40 CFR 796.3260 Aerobic Ready Biodegradability test (CO_2 evolution method) No 1992
Test system	Exposure Period: 28 Days and extended to 34 Days Inoculum: Activated sludge from municipal sewage wastewater treatment plant Kinetics: Reported
Test Conditions	Inoculum: activated sludge from municipal wastewater treatment plant Microbial density was 6.1x 10^3 CFU/ml; 1 flask Treated [medium + inoculum + test material (7.8 mg C/l)]; 1 flask Treated [medium + inoculum + test material (15.6 mg C/l)]; 1 flask Positive Control [medium + inoculum + sodium acetate (20 mg/l)]; 1 flask Blank Control[medium + inoculum] Procedure: Biodegradation experiments were performed in 3L test vessels containing medium solution, test substance and/or inoculum. Inoculum and medium solution were purged with CO_2-free air for 24 hours prior to addition of test material. The test system, containing 4 vessels, was carried out for 34 days at 21±2^{0}C, under a constant gas flow. The outgoing air from the biodegradation vessels was passed through CO_2-traps containing $Ba(OH)_2$ solutions. The amount of CO_2 produced during the course of the test was monitored, days 2, 5, 7, 9, 12, 15, 18, 25, 28 as well as day 30, 32, 34, 37. Biodegradation findings up to day 28 are reported in table below. Concentrations for Test Substance were 7.8 and 15.6 mg C /L Concentration for sodium acetate (positive control) was 20 mg C/L.
Results	**Biodegradation Results:** % Biodegradation [% of ThCO2]

Day	**2**	**5**	**7**	**9**	**12**	**15**	**18**	**22**	**25**	**28**
Conc (7.8 mg C/L)	5.1	23	39	42	49	58	64	68	68	68
Conc (15.6 mg C/L)	7.2	27	48	53	60	67	72	76	77	78
Mean Value ==>	**6.2**	**25**	**44**	**48**	**55**	**63**	**68**	**72**	**73**	**73**
Positive Control (sodium acetate)	18	33	46	50	55	67	77	83	85	85

Conclusions	Biodegradation occurred to the extent of 73% in 28 days for the test substance (mean of biodegradation at the two tested concentrations). The test substance considered to have met the "10-day window" criterion for "readily biodegradable".
Remarks	1) Limited report; no information on whether experiments were performed in dark, no information on stirring regime, amount of inoculum; pH, test medium solution, number of absorption bottles and the volume of $Ba(OH)_2$ used; the way of determination of CO_2-amount in the absorption traps; amount of total CO_2 evolution in the blank control 2) No replicates for test flasks, positive and for blank control flasks.

Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP and limited information in report. Number of replicates used.
References	Unpublished confidential business information
Other	Date last updated: December 12, 2003

Acute fish toxicity (CAS No. 57675-44-2)

Test Substance	9-Octadecenoic acid (Z)-, 2-ethyl-2-[(1-oxo-9-octadecenyl)oxy]methyl]-1,3-propanediyl ester, (Z)-
CAS Number	57675-44-2
Remarks	Purity was 100%
Method/guideline	OECD 203; EC L 383A/163-171 C 1 (1992)
Type (test type)	Acute fish toxicity study
Test System	Fish, freshwater
GLP	Yes
Year	1996
Species/Strain	Fish: Rainbow trout (*Oncorhynchus mykiss)*
Analyt. Monitoring	Analyses were performed by GC-FID
Exposure period	96 hours
Statist. Methods	Binomial probability analysis (Stephan *et al.*, 1978); probit or trimmed Spearman-Karber analyses were not applicable.
Test Conditions	96-hr static acute fish toxicity test at five nominal concentrations from 66 mg/L to 1056 mg/L Species: Rainbow trout (*Oncorhynchus mykiss*), mean length 34-38 mm Test performed in 40 L glass vessels containing 30 L well water (hardness 203 mg/L $CaCO_3$); 11.2-12.3°C; 16 h light/8h dark cycle; unfed; loading 0.37-0.48 g/L. The test substance (oil) was maintained as oil in water dispersion/suspension by a propeller (protected against the fish) above the system which created a vortex of 0.6-1.3 cm. No. of fish: 20/treatment Concentrations (nominal): 0 (untreated controls), 66, 132, 264, 528 and 1056 mg/L Physical Measurement: The pH, temperature and dissolved oxygen were performed daily. During course of 96 hr study, the pH ranged from 7.74 to 8.05; dissolved oxygen was between 7.5 to 9.6 mg/L; and temperature was 11.2-12.3°C. Observations: Mortality/symptoms at 24, 48, 72 and 96 hr GC limit of detection for test material was 1 mg/L.
Result	Nominal test conc. Loading Level (mg/L) — Mortality (96-hr) 0 Control (untreated) — 0 66 — 0 132 — 0 264 — 5 528 — 0 1056 — 0 No mortality was observed in the fish at nominal concentrations from 66 mg/L to 1056 mg/L except for one fish out of 20 (5%) at the 264 mg/L nominal exposure level.
Conclusion	The 96-h LC_{50} was >1056 mg/L (nominal concentration, oil in water suspension/dispersion). Nominal test concentrations were all above the water solubility of the test material

	(calculated to be 7.8 x 10^{-22} mg/L, EpiWin). GC-FID analysis revealed that test material was present in water samples (measured conc ranged from 4.2 to 166 mg/L). This is not unexpected since test material was mechanically dispersed in water. Hence, ecotoxicity data indicate that the test material would not be expected to cause acute toxicity in fish at its water saturation limit or water solubility limit (WSL).
Remarks	1) The fish were relatively small (34-38 mm, EC L 383 A: 60±20 mm). Since small fish may be more sensitive, this may be acceptable in a worst case approach. 2) Because the test substance is not soluble in water, it is kept in suspension by a propeller situated above the water surface, utilizing oil in water dispersion method. 3) The LC50 is determined using the nominal concentration, since test material was water-insoluble. 4) The temperature during the study was at the lower range of temperature recommended (11.2-12.3 °C versus EC L 383 A recommended 12-17°C).
Data Quality	Reliable with restrictions [Klimisch reliability 2].
References	Unpublished confidential business information.
Other	Date last updated: December 15, 2003.

Biodegradation (CAS No. 57675-44-2)

Test Substance	9-Octadecenoic acid (Z)-, 2-ethyl-2-[(1-oxo-9-octadecenyl)oxy]methyl]-1,3-propanediyl ester, (Z)-
CAS Number	57675-44-2
Remarks	Purity was 100%
Method/guideline	EPA 560/6-82-003 (equivalent to OECD 301B methodology) Shake Flask Aerobic Biodegradation - CO_2 evolution method using non-acclimated inoculum
Test type	Aerobic Biodegradation - CO_2 evolution method
GLP	No
Year	1993
Test system	Exposure Period: 28 Days Inoculum: Activated Sludge, Domestic, Unacclimated. Kinetics: Not Reported Biodegradation Products: Not Reported Analytical Monitoring: CO_2 evolution monitored in traps containing base solution.
Test Conditions	Inoculum: Activated sludge obtained from wastewater treatment plant. Amount inoculum added was sufficient to final inoculum solids conc. of 30 mg solids/L. Duplicate flasks Treated [medium + inoculum + test material (10 mg C/l)]; Duplicate flasks Positive Control [medium + inoculum + rapeseed oil (10 mg C/l))]; Duplicate Blank Control [medium + inoculum]. Incubation was performed under continuous shaking in 2L flasks, containing 1L of medium, test substance and/or inoculum at 25±3 ^{0}C in the dark. Evolved CO_2 was collected in appropriate trap containing 10 ml 0.2N KOH. CO_2 was monitored at various time points over a period of 28 days. Flask CO_2 traps were sampled at days 2, 5, 9, 14, 21 and 28. The amount of CO_2 was determined in the traps by back titration with 0.2N HCl, after addition of $Ba(Cl)_2$ and indicator. One day prior to the final sampling, the medium was acidified with 1 ml of concentrated sulfuric acid. Blank controls were used to subtract for background CO_2 production.

	Concentrations for Test Substance was 10 mg C /L. Concentration for rapeseed oil (positive control) was 10 mg C/L.
Results	**Biodegradation Results:** % Biodegradation [% of ThCO2] mean value (n=2)

Day	2	5	9	14	21	28
Test Material (10 mg C/L)	7.0	41.6	58.1	68.5	76.1	80.7
Positive Control (rapeseed oil 10 mg C/L)	14.0	49.1	65.8	74.1	79.2	79.4

	Test material met "10-day window" criteria for ready biodegradability. From the biodegradation time plot, the 10% mark was reached on Day 2.3 and 10 days later, on Day 12.3, the biodegradation was 64.9%. Positive controls achieved 79.4% biodegradation in 28 days and met the "readily biodegradable" criteria.
Conclusions	Biodegradation was 80.7% in 28 days. The test substance was readily biodegradable.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP. Test method used was essentially equivalent to OECD 301B test method.
References	Unpublished confidential business information
Other	Date last updated: December 15, 2003

Biodegradation (CAS No. 57675-44-2)

Test Substance	9-Octadecenoic acid (Z)-, 2-ethyl-2-[(1-oxo-9-octadecenyl)oxy]methyl]-1,3-propanediyl ester, (Z)-
CAS Number	57675-44-2
Remarks	Purity not indicated but was treated as 100% in study report.
Method/guideline	OECD 301B (1992), 92/69/EEC L383, C.4-C (1992)
Test type	Aerobic Ready Biodegradability test (Modified Sturm CO_2 evolution method)
GLP	Yes
Year	1995
Test system	Exposure Period: 28 Days Inoculum: Activated sludge from municipal sewage wastewater treatment plant Kinetics: Not Reported
Test Conditions	Inoculum: activated sludge from municipal wastewater treatment plant Amount of inoculum was 10 ml/L medium solution. Microbial density was not indicated. 2 flasks Treated [medium + inoculum + test material (15.4 mg C/l)]; 1 flask Positive Control [medium + inoculum + sodium acetate (11.7 mg C/l)]; 1 flask Toxicity Control [medium + inoculum + test material (15.4 mg C/l) + sodium acetate (11.7 mg C/l)]; 2 flasks Blank Control [medium + inoculum]
	Procedure: Biodegradation experiments were performed under continuous stirring in 2L brown glass bottles containing medium solution, test substance and/or inoculum. Inoculum and medium solution were purged (pre-acclimated) with CO_2-free air for 24 hours prior to addition of test material. After addition of materials, the test system was stirred, aerated with CO_2-free air at 21-23^{0}C for 28 days at constant gas flow. The outgoing air from the biodegradation vessels was passed through three consecutive traps containing 100 ml of 0.0125N $Ba(OH)_2$ solution. The amount of CO_2 produced during the course of the test was monitored at days 3, 5, 7, 10, 14, 17, 21, 24, 27, 28 (titrated on day 29). The amounts of

carbon dioxide in the Ba(OH)2 traps were determined by back-titrating residual base with 0.05M HCl. On day 28, HCl was added to the brown bottles where after the final titration was performed on day 29. The pH of the medium solution in the test systems was monitored at the start of the test and at the end on day 28, just prior to addition of the HCl. The pH value varied from 7.4 to 8.0 during the study. Biodegradation findings up to day 28 are reported in table below.

Concentration for Test Substance was 15.4 mg C /L
Concentration for sodium acetate (positive control) was 11.7 mg C/L.

Results

Biodegradation Results:

	% Biodegradation [% of ThCO2]									
Day	**3**	**5**	**7**	**10**	**14**	**17**	**21**	**24**	**27**	**28**
Test Flask 1	4.1	9.6	19	29	56	67	75	78	80	88
Test Flask 2	6.3	15	22	33	58	65	71	74	76	82
Mean Value ==>	**5.2**	**12.3**	**20.5**	**31**	**57**	**66**	**73**	**76**	**78**	**85**
Toxicity Control	4.0	8.3	22	32	48	57	63	67	68	70
Positive Control	19	35	50	62	70	73	76	81	84	97

Conclusions

Biodegradation occurred to the extent of 85% in 28 days for the test substance (mean of two flasks). Although the study report indicated that test substance technically did not meet the "10-day window" criterion for "readily biodegradable", it was very close to reaching the 60% mark. From the data in the table, it can be seen that at day 5, the biodegradation reached 12.3% (greater than 10% biodegradation); 10 days later on day 15, the biodegradation was estimated to be ca. 60%.

Remarks

1) Test material was extensively biodegraded (85% in 28 days) and may have just missed the >60% biodegradation mark within the "10-day window" for ready biodegradability.
2) Toxicity control results indicate that the test material is not inhibitory to microbial organism in the OECD 301B test system.
3) Slight deviation in temperature was noted but no significant impact on results expected.

Data Quality

Reliable without restrictions [Klimisch reliability 1].

References

Unpublished confidential business information

Other

Date last updated: December 15, 2003

Acute Oral Toxicity (CAS No. 67762-53-2)

Test Substance	Carboxylic acids, C5-9, tetraesters with pentaerythritol
CAS Number	67762-53-2
Remarks	Test material purity was 81% with remainder being comprised of 19% Carboxylic acids, C5-9, hexaesters with dipentaerythritol (CAS No. 67762-52-1)
Method/guideline	OECD 420
Test type	Acute oral toxicity
GLP	Yes
Year	1999
Test system	Species (Strain): Rats (Sprague-Dawley Crl:CD); weight: 287-349 g (males), 216-236 g (females), 9-12 weeks old Sex: Male and female No. of animals: 5 /sex/treatment Route: Single oral gavage Dosage: 1940 mg/kg b.w. or dose volume 2.0 ml/kg (undiluted) b.w.

	Statist. Methods: Not applicable
Test conditions	Five male and 5 female Sprague-Dawley rats were fasted ~18 hrs prior to dosing. Single oral (gavage) of 1940 mg/kg bw (dosing volume 2.00 ml/kg bw) was administered; no controls; feeding *ad libitum* about 4 hrs after dosing and throughout observation period. Observations: Mortality was observed twice daily for 14 days. Clinical signs were observed several times on the day 1 and daily until day 15. Body weights were measured on day 1, 8 and 15. Necropsy was performed on day 15
Results/Remarks	No mortality was observed in any of the female or male rats. There were no reports of any treatment-related effects on clinical signs of toxicity or body weight gain. There were no treatment-related effects, gross morphology or histopathology at necropsy. One male animal had unformed stool 4 hours after administration.
Conclusions	The oral LD50 was > 1940 mg/kg.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: December 15, 2003.

Repeated Dose Toxicity (CAS No. 67762-53-2)

Test Substance	Carboxylic acid, C5-9, tetraesters with pentaerythritol
CAS Number	67762-53-2
Remarks	Purity of 100%
Method/guideline	Other
Test type	13-Week Dermal Toxicity Study
GLP	No
Year	1985
Species/strain	Rat/Sprague Dawley [Crl: CD BR] ; age: 6.5 - 7 weeks
Route of Administ.	Dermally
Duration of test	13-weeks
No. of animals	10/sex/dose [Group 1 (0.0 mg/kg); Group 2 (800 mg/kg); Group 3 (2000 mg/kg)]
Dose/Conc. Levels	0, 800 and 2000 mg/kg/day
Sex	Males and females
Frequency of treatment	5-Days per week for 13-weeks
Experimental Groups	Group 1 (0.0 mg/kg); Group 2 (800 mg/kg); Group 3 (2000 mg/kg)]
Post-exposure observat.	No
Statist. Methods	Duncan's multiple range test; chi-square
Remarks on Test Conditions	Test article was applied to the backs of groups of 10 male and 10 female rats, 5-days per week for 13-weeks at the dose level of 800 or 2000 mg/kg/day. Test article was dispensed by to the clipped backs of animals, which was not covered. The rats were fitted with cardboard Elizabethan collars to minimize ingestion of the test article. A similar group of 10 males and 10 females served as controls; they were treated in the same manner but without treatment. Assessment for toxic responses included: body and organ weights, clinical observations, sperm, morphology, hematology, serum chemistry, urinalyses, gross necropsy, and histopathology.
Results	The dermal bioavailability of the test article was 2 to 6%. Males treated with test article at 2000 mg/kg/day weighed 10% less than the controls after 13 weeks; those treated at

	800 mg/kg/day weighed 7% less. No effect on body weight occurred in females. There were no other indications of systemic toxicity; i.e., no mortality or organ toxicity. It was not clear if the decreased growth represented toxicity, but the effect was so slight that the NOAEL is considered to be 2000 mg/kg/day. Minimal skin irritation, mostly flaking with slight erythema, was observed in males and females of both groups of treated animals. Microscopic examination of the skin revealed very minor epidermal hyperplasia and chronic inflammation of the dermis. No differences were seen in sperm morphology. Ovaries, testes, epididymides, uterus, and vagina showed no adverse affects.
Conclusions	The NOAEL was considered to be 2000 mg/kg/day.
Data Quality	Reliable with restrictions [Klimisch reliability 2]
References	Unpublished confidential business information.
Other	Date: December 16, 2003

Genetic Toxicity In Vitro (CAS No. 67762-53-2)

Test Substance	Carboxylic acids, C5-9, tetraesters with pentaerythritol
CAS Number	67762-53-2
Remarks	Test material purity was 81% with remainder being comprised of 19% Carboxylic acids, C5-9, hexaesters with dipentaerythritol (CAS No. 67762-52-1)
Method/guideline	Not indicated but procedures similar to OECD 471 guidelines
Type of Study	Bacterial Reverse Mutation Assay
Test System	Bacterial (*Salmonella - Escherichia coli*)
GLP	Yes
Year	1999
Species/Strain	*Salmonella typhimurium* / TA1535, TA1537, TA98, TA100 and *Escherichia coli* / WP2uvrA
Metab. Activation	Aroclor 1254-induced rat liver preparations (S9 mixture)
Concentrations	33.3, 100, 333, 1000, 3330, and 5000 µg/plate of the test material
Statist. Methods	Not specified but positive controls were run concurrently with test substance.
Test Conditions/ Remarks	Ethanol was used a vehicle (negative) control. Concurrent positive control materials were: 2-aminoanthracene (TA100, TA1535, TA1537, Wp2uvrA), benzo(a)pyrene (TA98), all with S9; sodium azide (TA100, TA1535), 2-nitrofluorene (TA98), 4-nitroquinoline-N-oxide (WP2 uvrA), ICR-191 (TA1537), all without S9. Procedures were similar to OECD 471 procedures.
Results	The test substance was <u>negative</u> for mutagenic activity in the four *Salmonella* tester strains and in the *E. coli* strain, with or without metabolic activation. No mutagenic activity was observed at concentrations ranging from 33.3 µg/plate to the highest concentration of 5000 µg/plate. The bacterial strains tested included *Salmonella typhimurium* strains TA1535, TA1537, TA98; TA100 and *Escherichia coli* strain WP2uvrA. The negative (ethanol vehicle) control and positive controls gave the appropriate responses as expected. Precipitate was observed at 333 µg/plate and above. This may indicate that test concentrations may be at solubility limit in ethanol/water in test. No appreciable toxicity was observed.
Conclusions	

	The test substance was not mutagenic, with or without metabolic activation in the *Salmonella-Escherichia coli* / Mammalian Microsome Reverse Mutation assay.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date last updated: December 29, 2003.

Genetic Toxicity In Vivo (CAS No. 67762-53-2)

Test Substance	Carboxylic acids, C5-9, tetraesters with pentaerythritol
CAS Number	67762-53-2
Remarks	Purity was 100%
Method/guideline	Other, similar to procedures in OECD 474
Type of Study	*In vivo* micronucleus assay
Test system	Bone marrow and peripheral blood cells
GLP	No
Year	1991
Species/Strain	Rat (Sprague Dawley), 10-weeks old
Sex	Male
No. of animals	10 controls and 10 experimental
Route of Administ.	Inhalation (aerosolized)
Doses/conc. levels	0 and 0.5 mg/L
Exposure period	Daily, 6-hours/day, five days per week for two weeks
Statist. Methods	ANOVA, Tukey's test, Sheffe's test, SAS (Statistical Analysis Systems), SAS GLM (General Linear Model)
Remarks on Test Conditions	Male rats inhaled aerosolized test material at a dose of 0.5 mg/L. The dose was administered daily, 6-hours/day, five days per week, for two weeks. At the end of this period, bone marrow cells were collected. Mature red blood cells (normochromatic erythrocytes, NCE) and immature red blood cells (polychromatic erythrocytes, PCE) were evaluated for cytotoxicity and micronucleus formations.
Results	The test article was not cytotoxic to red blood cell formation, nor did it induce a statistically significant increase in the formation of micronucleated PCEs and NCEs in the bone marrow of the rats exposed to the material.
Conclusions	The test article did not cause chromosome damage in this test.
Data Quality	Reliable without restrictions [Klimisch reliability 1]
References	Unpublished confidential business data.
Other	Date: December 16, 2003

Reproductive Toxicity /Developmental Toxicity (CAS No. 67762-53-2)

Test Substance	Carboxylic acids, C5-9, tetraesters with pentaerythritol
CAS Number	67762-53-2
Remarks	100% Purity
Method/guideline	EPA Proposed guidelines for health assessment of suspect developmental toxicants. Federal Register 49 (227) p.46325
Test type	Developmental Toxicity Screen in Rats
GLP	No
Year	1986
Species/strain	Rats/Sprague-Dawley (approximately 9 weeks old)
Route of Administ.	Dermal
Duration of test	20-Days
No. of animals	Three groups of 15 presumed-pregnant rats: Six groups of 10 males and 10 females Group 1 (control); Group 2 (800 mg/kg); and Group 3 (2000 mg/kg)
Dose/Conc. Levels	0.0, 800.0 or 2000.0 mg/kg/day
Sex	Females
Frequency of treatment	Daily on each gestation days 0-19
Control Group	15 Females for Group 1
Post-exposure observat.	None.
Statist. Methods	Fisher's Exact or Dunnett's test
Remarks on Test Conditions	During the mating period female rats which had not previously borne pups were placed with male rats in a ratio of 1:1 and observed daily for evidence of having engaged in breeding activity. Presumed-pregnant rats were distributed among three experimental groups: one dermal control, and two exposed groups. At the start of the dosing phase of the study, all of the experimental groups contained 15 presumed-pregnant females. All treatments for Groups 1-3 were preformed on each of gestation days 0-19, where designation as gestation day 0 followed detection of a vaginal plug (*in situ* or expelled) and spermatozoa in the vaginal lavage fluid. Test article was applied once daily to the clipped, intact dorsal skin of the rat. In no case were the application sites covered. To minimize ingestion of the test material, the rats were fitted with cardboard Elizabethan-style collars. All animals were monitored throughout gestation until sacrifice for 1.) changes in appearance, behavior, and excretory function, and 2.) signs of ill-health, mortality, or abortion. A pre-partum investigation on a variety of fetal and maternal parameters for each of the groups was undertaken to assess the influence of test article on reproductive performance.
Results	Administration of test article to the uncovered skin of collared rats at doses of 800 or 2000 mg/kg/day produced slight skin irritation (erythema and flaking) at the site of application. Neither maternal parameters (food consumption, body weight gain) monitored throughout gestation (days 0-19) nor reproductive parameters (number of implants, resorptions, or viable fetuses) were adversely affected at either of the dose levels tested. No evidence of teratogenicity (abnormal development) was observed during external examination of fetuses from pregnant dams exposed to test article. Mean fetal body weights and crown-rump distances were similar in all of the experimental groups.
Conclusions	Dermal administration of test article did not adversely affect parameters of reproductive performance during gestation, nor did it adversely affect *in utero* survival and development of concepti.
Data Quality	Reliable with restrictions [Klimisch reliability 2]

References	Unpublished confidential business information
Other	Date: December 16, 2003

Acute fish toxicity (CAS No. 67762-53-2)

Test Substance	Carboxylic acids, C5-9, tetraesters with pentaerythritol
CAS Number	67762-53-2
Remarks	Test material purity was 88% with remainder being comprised of 12% Carboxylic acids, C5-9, hexaesters with dipentaerythritol (CAS No. 67762-52-1)
Method/guideline	EC, L 251/146-154 C 1 (1984)
Type (test type)	Acute fish toxicity study
Test System	Fish, freshwater
GLP	No
Year	1993
Species/Strain	Fish: Rainbow trout (*Oncorhynchus mykiss)*
Analyt. Monitoring	No analysis was performed
Exposure period	96 hours
Statist. Methods	Binomial probability analysis (Stephan *et al.*, 1978)
Test Conditions	96-hr static acute fish toxicity test at five nominal concentrations from 97 mg/L to 5012 mg/L Species: Rainbow trout (*Oncorhynchus mykiss*), mean length: 28-31 mm Test performed in 40 L glass vessels containing 30 L well water (hardness 211 mg/L $CaCO_3$); 12±2°C; 16 h light/8h dark cycle; unfed; loading 0.2 g/L. The test substance (oil) was maintained as oil in water dispersion/suspension by a propeller (protected against the fish) above the system which created a vortex of 0.6-1.3 cm. No. of fish: 20/treatment Concentrations (nominal): 0 (untreated controls), 97, 517, 1002, 2005 and 5012 mg/L Physical Measurement: The pH, temperature and dissolved oxygen were performed daily. During course of 96 hr study, the pH was 8.2, dissolved oxygen was 84-94% of saturation, and temperature was 11-12°C. Observations: Mortality/symptoms at 24, 48, 72 and 96 hr. Due to cloudiness at three highest doses, observation for mortality could not be made until end of study at 96 hr
Result	Nominal test conc. Loading Level (mg/L) — Mortality (96-hr) 0 Control (untreated) — 0 97 — 0 517 — 0 1002 — 0 2005 — 0 5012 — 0 No mortality was observed in the fish at nominal concentrations from 97 mg/L to 5012 mg/L.
Conclusion	The 96-h LC_{50} was >5012 mg/L (nominal concentration, oil in water suspension/dispersion). Nominal test concentrations were all above the water solubility of the test material (calculated to be 8.4 x 10^{-8} mg/L mg/L, EpiWin). Hence, ecotoxicity data indicate that the test material would not be expected to cause acute toxicity in fish at its water saturation limit or water solubility limit (WSL).

Remarks	1) The fish were relatively small (30 mm, EC L 383 A: 60±20 mm). Since small fish may be more sensitive, this may be acceptable in a worst case approach. 2) Because the test substance is not soluble in water, it was kept in suspension by a propeller situated above the water surface, utilizing an oil-in-water dispersion method. 3) The LC50 is determined using the nominal concentration, since test material has a very low water solubility. 4) The temperature during the study was at the lower range of temperature recommended (11-12°C versus EC L 383 A recommended 12-17°C).
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP and no chemical analysis data.
References	Unpublished confidential business information.
Other	Date last updated: December 29, 2003.

Biodegradation (CAS No. 67762-53-2)

Test Substance **CAS Number** **Remarks**	Carboxylic acids, C5-9, tetraesters with pentaerythritol 67762-53-2 Test material purity was 88% with remainder being comprised of 12% Carboxylic acids, C5-9, hexaesters with dipentaerythritol (CAS No. 67762-52-1)
Method/guideline	EPA 560/6-82-003 (equivalent to OECD 301B methodology) Shake Flask Aerobic Biodegradation - CO_2 evolution method using non-acclimated inoculum
Test type **GLP** **Year**	Aerobic Biodegradation - CO_2 evolution method No 1993
Test system	Exposure Period: 28 Days Inoculum: Activated Sludge, Domestic, Unacclimated. Kinetics: Not Reported Biodegradation Products: Not Reported Analytical Monitoring: CO_2 evolution monitored in traps containing base solution.
Test Conditions	Inoculum: Activated sludge obtained from wastewater treatment plant. Amount inoculum added was sufficient to final inoculum solids conc. of 30 mg solids/L. Duplicate flasks Treated [medium + inoculum + test material (10 mg C/l)]; Duplicate flasks Positive Control [medium + inoculum + rapeseed oil (10 mg C/l))]; Duplicate Blank Control [medium + inoculum]. Incubation was performed under continuous shaking in 2L flasks, containing 1L of medium, test substance and/or inoculum at 25±3 ^{0}C in the dark. Evolved CO_2 was collected in appropriate trap containing 10 ml 0.2N KOH. CO_2 was monitored at various time points over a period of 28 days. Flask CO_2 traps were sampled at days 2, 5, 9, 14, 21 and 28. In addition, biodegradation was monitored past the conventional 28 day period and the flask CO_2 traps were sampled also at days 36, 43, 57, 72 and 86. The amount of CO_2 was determined in the traps by back titration with 0.2N HCl, after addition of $Ba(Cl)_2$ and indicator. Blank controls were used to subtract for background CO_2 production. Concentrations for Test Substance was 10 mg C /L for test substance. Concentration for rapeseed oil (positive control) was 10 mg C/L.

Results	**Biodegradation Results:** % Biodegradation [% of ThCO2] mean value **Day 2 5 9 14 21 28** Test Material (10 mg C/L) 2.1 16.6 25.1 34.8 41.6 47.1 Positive Control (rapeseed oil, 10 mg C/L) 17.7 53.2 65.5 71.4 74.3 79.0 Test material did not meet "10-day window" criteria for ready biodegradability. Positive controls achieved 79.0% biodegradation in 28 days and met the "readily biodegradable" criteria. Biodegradation monitoring was continued beyond the conventional 28 day period and the results are summarized below: **Biodegradation Results (past 28 days):** % Biodegradation [% of ThCO2] mean value **Day 28 36 43 57 72 86** Test Material (10 mg C/L) 47.1 51.7 62.7 75.4 79.0 85.9 Positive Control (rapeseed oil, 10 mg C/L) 79.0 79.0 79.0 80.6 80.6 82.6
Conclusions	Biodegradation was 47.1% in 28 days. The test substance was not readily biodegradable. Continued monitoring of biodegradation (CO_2 evolution) showed that test material could be biodegraded to the extent of 85.9% in 86 days.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP. Test method used was essentially equivalent to OECD 301B test method. Temperature was carried out at ambient temperature.
References	Unpublished confidential business information
Other	Date last updated: December 29, 2003

Acute Oral Toxicity (CAS No. 68424-31-7)

Test Substance	Fatty acids, C5-10, esters with pentaerythritol
CAS Number	68424-31-7
Remarks	Purity was approximately 100%
Method/guideline	84/449/EEC B1.
Test type	Acute oral toxicity
GLP	Yes
Year	1987
Test system	Species (Strain): Rat (Wistar); weight: 205-224 g (males), 161-179 g, g (females), 7 weeks old Sex: Male and female No. of animals: 5 /sex/treatment Route: Single oral gavage Dosage: 5000 mg/kg bw (dosing volume 5.5 ml/kg) Statist. Methods: Not applicable

Test conditions	Five male and 5 female rats were fasted overnight prior to dosing. Single oral (gavage) of 5000 mg/kg b.w. was administered; no controls; feeding *ad libitum* about 3-4 hrs after dosing and throughout observation period. Observations: Mortality / clinical signs were observed several times on day 0 (day of dosing) and daily until day 14. Body weights on day 0, 7 and 14. Necropsy on day 14.
Results/Remarks	No mortality was observed in any of the female or male rats. There were no reports of any treatment-related effects on clinical signs of toxicity or body weight gain. There were no treatment-related adverse effects, gross morphology or histopathology at necropsy.
Conclusions	The oral LD50 was > 5000 mg/kg b.w.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date last updated: December 30, 2003.

Acute toxicity to aquatic plants (e.g., algae) (CAS No. 68424-31-7)

Test Substance	Fatty acids, C5-10, esters with pentaerythritol
CAS Number	68424-31-7
Remarks	Purity was not indicated. Test material was mixture of CAS No. 68424-31-7 (fatty acids, C5-10, esters with pentaerythritol) and CAS No. 70983-72-1 (fatty acids, C5-10 esters with dipentaerythritol); composition of PE and diPE esters of C5-10 fatty acids was not specified.
Method/guideline	OECD 201, EEC L383A C3 (1992), ISO 8692:1989(E)
Type (test type)	Algae, growth inhibition test
Test System	Aquatic plant (e.g., algae)
GLP	Yes
Year	1996
Species/Strain	Green algae / *Scenedesmus subspicatus*
Analyt. Monitoring	Analyses were performed (GC-FID quantitation)
Exposure period	72 hours
Statist. Methods	Not specified
Test Conditions/ Remarks	Static 72 hr algae growth inhibition study Species: Green algae (*Scenedesmus subspicatus*) Tests were performed in containers with algal medium under continuous illumination and agitation (shaker). Initial Cell Conc.: 8.2 x 10^3 cells/mL No. of replicates: 3 per treatment, 6 for controls Concentrations (nominal): 0 (untreated controls), 1.0, 1.8, 3.2, 5.6 and 10 mg/L (as dispersions) Physical Measurements: The pH and temperature were performed. The range of pH was 6.8-7.9 in the test solutions and temperature was 21-24 °C. Observations: Cell density at 0, 24, 48 and 72 hr by particle counting. Chemical analyses of test material were carried out by solvent extraction from collected water samples (one replicate per treatment at 0 and 72 hr) and quantitated by GC/FID. Mean measured conc are given below in table

Results		Nominal Concentrations of Dispersion Water Solutions Tested (mg/L)					
		0 (Control)	1.0	1.8	3.2	5.6	10
		Mean Measured Concentrations (mg/L)					
Parameter	**Time (hr)**	**0**	**0.60**	**0.84**	**1.8**	**2.4**	**4.4**
Mean cell density [10^4 cells/ml]	0	1	1	1	1	1	1
	24	4	3	3	4	4	3
	48	15	15	15	17	14	20
	72	68	67	70	88	70	101
% Inhibition - AUC	0-72	0	3	-2	-26	-1	-42
% Inhib.-growth rate	0-72	0	1	-5	-6	-3	-17

Remark/comment	1) In the report no information is available about the light regime and intensity. Since no effect on the control cell growth was seen, the circumstances during the study can expected to be correct, or at least acceptable to create a valid test. 2) The growth inhibition was calculated according to the method recommended in the OECD 201 test guidelines.
Conclusions	72-hr EC_{50} was estimated to be > 4.4 mg/L (measured water concentration) Test material was shown to be present in water test solutions and measured levels were above the water solubility limit or water-saturated levels (WSL) of the test material. However, the material was dispersed in test solutions and the measured concentrations are likely to include oil droplet dispersions of the test material and not truly reflect the water solubilized fraction. The data may suggest that the test material would not be expected to cause aquatic toxicity below or close to its water saturation levels or water solubility limits (WSL).
Data Quality	Reliable without restrictions [Klimisch reliability 1]. 72-hr EC50 value was based on measured concentrations and not nominal loading rates. .
References	Unpublished confidential business information.
Other	Date last updated: January 8, 2004.

Acute Oral Toxicity (CAS No. 68424-34-0)

Test Substance	Fatty acids, C5-10, mixed esters with pentaerythritol and valeric acid
CAS Number	68424-34-0
Remarks	Purity was not indicated
Method/guideline	Not indicated
Test type	Acute oral toxicity
GLP	No
Year	1982
Test system	Species (Strain) Rat (Wistar); weight: 200-233 g (males), Sex: Male No. of animals: 10 male/treatment Route: Single oral gavage Dosage: 5000 mg/kg bw; no controls Statist. Methods: Not applicable
Test conditions	Single oral (gavage) administration of 5.0 g/kg to fasted male rats (~16-20 h prior to dosing) Observations: Mortality/clinical signs 3-4 hours post dose and daily until day 14. Body weights on day 0 and 14. Necropsy on day 14
Results/Remarks	No mortality was observed in any animals. There were no reports of any treatment-related effects or body weight gain. There were no treatment-related adverse effects, gross morphology or histopathology at necropsy. Minor clinical observations included chromodacryorrhea, ptosis and piloerection
Conclusions	The oral LD50 was > 5000 mg/kg bw in male rats
Data Quality	Reliable with restrictions [Klimisch reliability 2]. The report was limited. No female rats were evaluated and body weights should have been determined every week.
References	Unpublished confidential business information.
Other	Date last updated: December 10, 2003.

Acute Oral Toxicity (CAS No. 68648-28-2)

Test Substance	Linseed oil, ester with pentaerythritol
CAS Number	68648-28-2
Remarks	Purity not indicated
Method/guideline	OECD 401
Test type	Acute oral toxicity
GLP	Yes
Year	1999
Test system	Species (Strain): Rats (Sprague-Dawley), age 49-74 days-old Sex: Male and female; weight: 222-235 g (males), 211-229 g (females) No. of animals: 5 /sex/treatment Route: Single oral gavage Dosage: 2000 mg/kg (dosing volume 2 ml/kg, undiluted) Statist. Methods: None required
Test conditions	Five male and 5 female Sprague-Dawley rats were fasted overnight and dosed by oral gavage with 2000 mg/kg body weight of the test material. No controls; feeding and water *ad libitum* hr after dosing. Observations for mortality and clinical manifestations were carried out daily for 14 days. Observations included changes in skin and fur, eyes and mucous membranes, and respiratory, circulatory, autonomic and central nervous system and somatomotor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. Body weights were measured on day 0, 1, 7 and 14. Gross necropsy was performed on all animals sacrificed on day 14.
Results/Remarks	No mortality or clinical signs of toxicity were observed in any of the female or male rats. There were no treatment-related body weight changes. No abnormalities or gross lesions were observed at necropsy. Clinical observations and necropsy observations were normal.
Conclusions	The oral LD50 was > 2000 mg/kg.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: May 28, 2004.

Genetic Toxicity In Vitro (CAS No. 68648-28-2)

Test Substance	Linseed oil, ester with pentaerythritol
CAS Number	68648-28-2
Remarks	Purity not specified.
Method/guideline	OECD 471 (1997)
Type of Study	Bacterial Reverse Mutation Assay
Test System	Bacterial (*Salmonella - Escherichia coli*)
GLP	Yes
Year	2003
Species/Strain	*Salmonella typhimurium* / TA98, TA100, TA1535 and TA1537 and *Escherichia coli* / WP2 (Moltox, Inc., Boone, NC)
Metab. Activation	Aroclor 1254-induced Sprague-Dawley rat liver preparations (S9 mixture)
Concentrations	1, 10, 100, 1000, 10,000 and 100,000 µg/plate of the test material (without S9 mix)

	1, 10, 100, 1000, 10,000 and 100,000 µg/plate of the test material (with S9 mix) A density of 1 gm/ml was cited for the test material in the report. Test material was tested neat and at 10, 100, 1000, 10000 and 10^5-fold dilution in DMSO. Each neat or dilution solution of the test material was evaluated at 100 µl/plate
Statist. Methods	ANOVA (analysis of variance) and Newman-Keuls test for confirmation of pairwise comparison. Positive and negative controls were run concurrently with test substance.
Test Conditions/ Remarks	DMSO was used a vehicle (negative) control. Concurrent positive control materials were 2-aminoanthracene (all strains with S9); 2-nitrofluorene (TA98), sodium azide (TA100, TA1535), 9-aminoacridine (TA1537), 1-ethyl-3-nitro-1-nitrosoguanidine (ENNG) (*E. coli* WP2) (without S9)
Results	The test substance was negative for mutagenic activity in the four *Salmonella* tester strains and in the *E. coli* strain, with or without metabolic activation. No mutagenic activity was observed at concentrations ranging from 1µg/plate to the highest concentration of 100,000 µg/plate (neat). The bacterial strains tested included *Salmonella typhimurium* strains TA98; TA100, TA1535 and TA1537; and *Escherichia coli* strain WP2. The negative (vehicle) control and positive controls gave the appropriate responses as expected.
Conclusions	The test substance was <u>not</u> mutagenic, with or without metabolic activation in the *Salmonella-Escherichia coli* / Mammalian Microsome Reverse Mutation assay.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: June 1, 2004.

Genetic Toxicity In Vitro (CAS No. 68648-28-2)

Test Substance	Linseed oil, ester with pentaerythritol
CAS Number	68648-28-2
Remarks	Purity not indicated
Method/guideline	OECD 473 (1997 Guideline)
Type of Study	In Vitro Mammalian Chromosomal Aberration Test
Test System	Chinese hamster ovary (CHO) cell line
GLP	Yes
Year	2003
Species/ cell type	CHO cells
Metab. activation	Arochlor 1254-induced Sprague-Dawly rat liver S9 mixture
Concentrations	0.05, 0.5 and 5 µl/ml (approx. 50, 500 and 5000 µg/ml based on a density of 1.0 gm/ml) Negative vehicle control was Ham's F-12 complete medium
Statist. Methods	Statistical significance was determined by the chi-square test
Test Conditions /Remarks	Study was carried out to assess the ability of test substance to induce chromosomal aberrations in CHO cells cultured in vitro. Negative and positive control cultures were also prepared. One hour before the end of the incubation period, cell division was arrested with Colemid, the cells harvested and slides prepared so that the metaphase cells could be examined for chromosomal damage. Negative Control: Ham's F-12 complete medium Positive Controls: mitomycin-C (-S9), cyclophosphamide (+S9)

Results	0.05, 0.5 and 5 µl/ml dose levels 1) without S9: 18 h exposure. 3) with S9: 2 h exposure + 16 h recovery. Colcemid was added 1 hr prior to harvesting			
Exposure (h)	Metabolic activation	Doses tested [µl/ml]	Aberrations [%] at doses, respectively	Test result
2	With	0.05, 0.5 and 5	0.5. 1.0 and 3.0	Negative
18	Without	0.05, 0.5 and 5	0.0, 2.0 and 3.5	Negative
Remark/comment	1) The positive and negative controls gave the expected responses to fulfill the requirements of a valid test. 2) Negative control (Ham's F-12 complete medium) gave 1.0% aberrations with and without metabolic activation. Positive control (cyclophosphamide) produced 18% aberrations in the metabolically activated CHO cell assay. Positive control (mitomycin C) produced 14% aberrations in the non-activated CHO cell assay.			
Conclusions	The test material is not clastogenic in the CHO cell culture test system, with or without metabolic activation. Regardless of dose level (from 0.05 µl/ml to as high as 5 µl/ml) and dosing regimen, the test substance was concluded to be negative for structural and numerical chromosome aberrations, with or without S-9.			
Data Quality	Reliable without restrictions [Klimisch reliability 1].			
References	Unpublished confidential business information.			
Other	Date: June 2, 2003.			

Acute Oral Toxicity (CAS No. 70983-72-1)

Test Substance	Fatty acids, C5-10, esters with dipentaerythritol
CAS Number	70983-72-1
Remarks	Purity was not indicated
Method/guideline	Not indicated
Test type	Acute oral toxicity
GLP	No
Year	1982
Test system	Species (Strain) Rat (Wistar); weight: 200-224 g Sex: Male No. of animals: 10 males/treatment Route: Single oral gavage Dosage: 5000 mg/kg bw (dosing volume 0.97-1.0 ml) Statist. Methods: Not applicable
Test conditions	Ten male rats were fasted (~16-20 hrs) overnight prior to dosing. Single oral (gavage) of 5000 mg/kg b.w. was administered; no controls; feeding *ad libitum* about 3-4 hrs after dosing and throughout observation period. Observations: Mortality / clinical signs were observed 3 to 4 hrs after dosing and daily thereafter until day 14. Body weights on day 0 and 14. Necropsy on day 14.
Results/Remarks	No mortality was observed in any of the male rats. There were no reports of any treatment-related effects on clinical signs of toxicity or body weight gain. There were no treatment-related adverse effects, gross morphology or histopathology at necropsy. Some clinical observations included chromodacryorrhea, piloerection, anogenital area wet or stained yellow and respiratory rattle during one day
Conclusions	The oral LD50 was > 5000 mg/kg b.w.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP and limited report. Only male rats were used and body weights were not performed on day 7.
References	Unpublished confidential business information.
Other	Date last updated: January 9, 2004.

Acute toxicity to aquatic plants (e.g., algae) (CAS No. 70983-72-1)

Test Substance	Fatty acids, C5-10, esters with dipentaerythritol
CAS Number	70983-72-1
Remarks	Purity was not indicated. Test material was mixture of CAS No. 70983-72-1 (fatty acids, C5-10 esters with dipentaerythritol) and CAS No. 68424-31-7 (fatty acids, C5-10, esters with pentaerythritol); composition of diPE and PE esters of C5-10 fatty acids was not specified.
Method/guideline	OECD 201, EEC L383A C3 (1992), ISO 8692:1989(E)
Type (test type)	Algae, growth inhibition test
Test System	Aquatic plant (e.g., algae)
GLP	Yes
Year	1996
Species/Strain	Green algae / *Scenedesmus subspicatus*

Analyt. Monitoring	Analyses were performed (GC-FID quantitation)
Exposure period	72 hours
Statist. Methods	Not specified
Test Conditions/ Remarks	Static 72 hr algae growth inhibition study Species: Green algae (*Scenedesmus subspicatus*) Tests were performed in containers with algal medium under continuous illumination and agitation (shaker). Initial Cell Conc.: 8.2 x 10^3 cells/mL No. of replicates: 3 per treatment, 6 for controls Concentrations (nominal): 0 (untreated controls), 1.0, 1.8, 3.2, 5.6 and 10 mg/L (as dispersions) Physical Measurements: The pH and temperature were performed. The range of pH was 6.8-7.9 in the test solutions and temperature was 21-24 °C. Observations: Cell density at 0, 24, 48 and 72 hr by particle counting. Chemical analyses of test material were carried out by solvent extraction from collected water samples (one replicate per treatment at 0 and 72 hr) and quantitated by GC/FID. Mean measured conc are given below in table

Results

Nominal Concentrations of Dispersion Water Solutions Tested (mg/L)

		0 (Control)	**1.0**	**1.8**	**3.2**	**5.6**	**10**
		Mean Measured Concentrations (mg/L)					
Parameter	**Time (hr)**	**0**	**0.60**	**0.84**	**1.8**	**2.4**	**4.4**
Mean cell density [10^4 cells/ml]	0	1	1	1	1	1	1
	24	4	3	3	4	4	3
	48	15	15	15	17	14	20
	72	68	67	70	88	70	101
% Inhibition - AUC	0-72	0	3	-2	-26	-1	-42
% Inhib.-growth rate	0-72	0	1	-5	-6	-3	-17

Remark/comment	1) In the report no information is available about the light regime and intensity. Since no effect on the control cell growth was seen, the circumstances during the study can expected to be correct, or at least acceptable to create a valid test. 2) The growth inhibition was calculated according to the method recommended in the OECD 201 test guidelines.
Conclusions	72-hr EC_{50} was estimated to be > 4.4 mg/L (measured water concentration) Test material was shown to be present in water test solutions and measured levels were above the water solubility limit or water-saturated levels (WSL) of the test material. However, the material was dispersed in test solutions and the measured concentrations are likely to include oil droplet dispersions of the test material and not truly reflect the water solubilized fraction. The data may suggest that the test material would not be expected to cause aquatic toxicity below or close to its water saturation levels or water solubility limits (WSL).
Data Quality	Reliable without restrictions [Klimisch reliability 1]. 72-hr EC50 value was based on measured concentrations and not nominal loading rates. .
References	Unpublished confidential business information.
Other	Date last updated: January 9, 2004.

Acute Oral Toxicity (CAS No. 67762-52-1)

Test Substance	Carboxylic acids, C5-9, hexaesters with dipentaerythritol
CAS Number	67762-52-1
Remarks	Test material purity was 19% with remainder being comprised of 81% carboxylic acids, C5-9, tetraesters with pentaerythritol (CAS No. 67762-53-2)
Method/guideline	OECD 420
Test type	Acute oral toxicity
GLP	Yes
Year	1999
Test system	Species (Strain) Rats (Sprague-Dawley Crl:CD); weight: 287-349 g (males), 216-236 g (females), 9-12 weeks old Sex: Male and female No. of animals: 5 /sex/treatment Route: Single oral gavage Dosage: 1940 mg/kg b.w. or dose volume 2.0 ml/kg (undiluted) b.w. Statist. Methods: Not applicable
Test conditions	Five male and 5 female Sprague-Dawley rats were fasted ~18 hrs prior to dosing. Single oral (gavage) of 1940 mg/kg bw (dosing volume 2.00 ml/kg bw) was administered; no controls; feeding *ad libitum* about 4 hrs after dosing and throughout observation period. Observations: Mortality was observed twice daily for 14 days. Clinical signs were observed several times on the day 1 and daily until day 15. Body weights were measured on day 1, 8 and 15. Necropsy was performed on day 15
Results/Remarks	No mortality was observed in any of the female or male rats. There were no reports of any treatment-related effects on clinical signs of toxicity or body weight gain. There were no treatment-related effects, gross morphology or histopathology at necropsy. One male animal had unformed stool 4 hours after administration.
Conclusions	The oral LD50 was > 1940 mg/kg.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: December 15, 2003.

Genetic Toxicity In Vitro (CAS No. 67762-52-1)

Test Substance	Carboxylic acids, C5-9, hexaesters with dipentaerythritol
CAS Number	67762-52-1
Remarks	Test material purity was 19% with remainder being comprised of 81% carboxylic acids, C5-9, tetraesters with pentaerythritol (CAS No. 67762-53-2)
Method/guideline	Not indicated but procedures similar to OECD 471 guidelines
Type of Study	Bacterial Reverse Mutation Assay
Test System	Bacterial (*Salmonella - Escherichia coli*)
GLP	Yes
Year	1999
Species/Strain	*Salmonella typhimurium* / TA1535, TA1537, TA98, TA100

	and *Escherichia coli* / WP2uvrA
Metab. Activation	Aroclor 1254-induced rat liver preparations (S9 mixture)
Concentrations	33.3, 100, 333, 1000, 3330, and 5000 μg/plate of the test material
Statist. Methods	Not specified but positive controls were run concurrently with test substance.
Test Conditions/ Remarks	Ethanol was used a vehicle (negative) control. Concurrent positive control materials were: 2-aminoanthracene (TA100, TA1535, TA1537, Wp2uvrA), benzo(a)pyrene (TA98), all with S9; sodium azide (TA100, TA1535), 2-nitrofluorene (TA98), 4-nitroquinoline-N-oxide (WP2 uvrA), ICR-191 (TA1537), all without S9. Procedures were similar to OECD 471 procedures.
Results	The test substance was negative for mutagenic activity in the four *Salmonella* tester strains and in the *E. coli* strain, with or without metabolic activation. No mutagenic activity was observed at concentrations ranging from 33.3 μg/plate to the highest concentration of 5000 μg/plate. The bacterial strains tested included *Salmonella typhimurium* strains TA1535, TA1537, TA98; TA100 and *Escherichia coli* strain WP2uvrA. The negative (ethanol vehicle) control and positive controls gave the appropriate responses as expected. Precipitate was observed at 333 μg/plate and above. This may indicate that test concentrations may be at solubility limit in ethanol/water in test. No appreciable toxicity was observed.
Conclusions	The test substance was not mutagenic, with or without metabolic activation in the *Salmonella-Escherichia coli* / Mammalian Microsome Reverse Mutation assay.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date last updated: December 29, 2003.

Acute fish toxicity (CAS No. 67762-52-1)

Test Substance	Carboxylic acids, C5-9, hexaesters with dipentaerythritol
CAS Number	67762-52-1
Remarks	Test material purity was 12% with remainder being comprised of 88% carboxylic acids, C5-9, tetraesters with pentaerythritol (CAS No. 67762-53-2)
Method/guideline	EC, L 251/146-154 C 1 (1984)
Type (test type)	Acute fish toxicity study
Test System	Fish, freshwater
GLP	No
Year	1993
Species/Strain	Fish: Rainbow trout (*Oncorhynchus mykiss)*
Analyt. Monitoring	No analysis was performed
Exposure period	96 hours
Statist. Methods	Binomial probability analysis (Stephan *et al.*, 1978)
Test Conditions	96-hr static acute fish toxicity test at five nominal concentrations from 97 mg/L to 5012 mg/L Species: Rainbow trout (*Oncorhynchus mykiss*), mean length: 28-31 mm Test performed in 40 L glass vessels containing 30 L well water (hardness 211 mg/L $CaCO_3$); 12±2°C; 16 h light/8h dark cycle; unfed; loading 0.2 g/L. The test substance (oil) was maintained as oil in water dispersion/suspension by a propeller (protected against the fish) above the system which created a vortex of 0.6-1.3 cm. No. of fish: 20/treatment

	Concentrations (nominal): 0 (untreated controls), 97, 517, 1002, 2005 and 5012 mg/L Physical Measurement: The pH, temperature and dissolved oxygen were performed daily. During course of 96 hr study, the pH was 8.2, dissolved oxygen was 84-94% of saturation, and temperature was 11-12°C. Observations: Mortality/symptoms at 24, 48, 72 and 96 hr. Due to cloudiness at three highest doses, observation for mortality could not be made until end of study at 96 hr
Result	Nominal test conc. Loading Level (mg/L) — Mortality (96-hr) 0 Control (untreated) — 0 97 — 0 517 — 0 1002 — 0 2005 — 0 5012 — 0 No mortality was observed in the fish at nominal concentrations from 97 mg/L to 5012 mg/L.
Conclusion	The 96-h LC_{50} was >5012 mg/L (nominal concentration, oil in water suspension/dispersion). Nominal test concentrations were all above the water solubility of the test material (calculated to be 8.4 x 10^{-8} mg/L mg/L, EpiWin). Hence, ecotoxicity data indicate that the test material would not be expected to cause acute toxicity in fish at its water saturation limit or water solubility limit (WSL).
Remarks	1) The fish were relatively small (30 mm, EC L 383 A: 60±20 mm). Since small fish may be more sensitive, this may be acceptable in a worst case approach. 2) Because the test substance is not soluble in water, it was kept in suspension by a propeller situated above the water surface, utilizing an oil-in-water dispersion method. 3) The LC50 is determined using the nominal concentration, since test material has a very low water solubility. 4) The temperature during the study was at the lower range of temperature recommended (11-12°C versus EC L 383 A recommended 12-17°C).
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP and no chemical analysis data.
References	Unpublished confidential business information.
Other	Date last updated: December 9, 2003.

Biodegradation (CAS No. 67762-52-1)

Test Substance	Carboxylic acids, C5-9, hexaesters with dipentaerythritol
CAS Number	67762-52-1
Remarks	Test material purity was 12% with remainder being comprised of 88% carboxylic acids, C5-9, tetraesters with pentaerythritol (CAS No. 67762-53-2)
Method/guideline	EPA 560/6-82-003 (equivalent to OECD 301B methodology) Shake Flask Aerobic Biodegradation - CO_2 evolution method using non-acclimated inoculum
Test type	Aerobic Biodegradation - CO_2 evolution method
GLP	No
Year	1993
Test system	Exposure Period: 28 Days

	Inoculum: Activated Sludge, Domestic, Unacclimated. Kinetics: Not Reported Biodegradation Products: Not Reported Analytical Monitoring: CO_2 evolution monitored in traps containing base solution.
Test Conditions	Inoculum: Activated sludge obtained from wastewater treatment plant. Amount inoculum added was sufficient to final inoculum solids conc. of 30 mg solids/L. Duplicate flasks Treated [medium + inoculum + test material (10 mg C/l)]; Duplicate flasks Positive Control [medium + inoculum + rapeseed oil (10 mg C/l))]; Duplicate Blank Control [medium + inoculum]. Incubation was performed under continuous shaking in 2L flasks, containing 1L of medium, test substance and/or inoculum at 25±3 ^{0}C in the dark. Evolved CO_2 was collected in appropriate trap containing 10 ml 0.2N KOH. CO_2 was monitored at various time points over a period of 28 days. Flask CO_2 traps were sampled at days 2, 5, 9, 14, 21 and 28. In addition, biodegradation was monitored past the conventional 28 day period and the flask CO_2 traps were sampled also at days 36, 43, 57, 72 and 86. The amount of CO_2 was determined in the traps by back titration with 0.2N HCl, after addition of $Ba(Cl)_2$ and indicator. Blank controls were used to subtract for background CO_2 production. Concentrations for Test Substance was 10 mg C /L for test substance. Concentration for rapeseed oil (positive control) was 10 mg C/L.
Results	**Biodegradation Results:** % Biodegradation [% of ThCO2] mean value

Day	**2**	**5**	**9**	**14**	**21**	**28**
Test Material (10 mg C/L)	2.1	16.6	25.1	34.8	41.6	47.1
Positive Control (rapeseed oil, 10 mg C/L)	17.7	53.2	65.5	71.4	74.3	79.0

Test material did not meet "10-day window" criteria for ready biodegradability. Positive controls achieved 79.0% biodegradation in 28 days and met the "readily biodegradable" criteria.

Biodegradation monitoring was continued beyond the conventional 28 day period and the results are summarized below:

Biodegradation Results (past 28 days):

% Biodegradation [% of ThCO2] mean value

Day	**28**	**36**	**43**	**57**	**72**	**86**
Test Material (10 mg C/L)	47.1	51.7	62.7	75.4	79.0	85.9
Positive Control (rapeseed oil, 10 mg C/L)	79.0	79.0	79.0	80.6	80.6	82.6

Conclusions	Biodegradation was 47.1% in 28 days. The test substance was not readily biodegradable. Continued monitoring of biodegradation (CO_2 evolution) showed that test material could be biodegraded to the extent of 85.9% in 86 days.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Not GLP. Test method used was essentially equivalent to OECD 301B test method. Temperature was carried out at ambient temperature.
References	Unpublished confidential business information
Other	Date last updated: December 29, 2003

Part II. Surrogate Polyol Esters

Melting Point, Boiling Point, Vapor Pressure, Partition Coefficient, Water Solubility (CAS No. 189120-64-7) Trimethylolpropane esters of heptanoic and octanoic acid - Surrogate Polyol Ester

Test Substance **CAS Number** **Remarks**	Trimethylolpropane esters of heptanoic and octanoic acid 189120-64-7 Purity was 100%		
	GLP (Yes/No)	**METHOD/ GUIDELINE**	**RESULTS / CONCLUSIONS**
Physicochemical Properties			
Melting Point/ Freezing Point	Yes	OECD 102	< -25 °C
Boiling Point	Yes	OECD 103	> 310 °C (not determinable, decomposes at temp above 310°C without boiling)
Vapor Pressure	Yes	OECD 104	3.5×10^{-6} Pascals at 25 °C
Partition Coeffic.	Yes	OECD 107/117	log P > 7
Water Solubility	Yes	OECD 105	Less than 0.1 mg/L (based on GC analysis and limit of detection)
Year	2000		
Remarks	Determination of a complete battery of physicochemical properties for the test substance CAS No. 189120-64-7, including those designated above has been carried out under GLP and by methods, which are in compliance with the OECD and EEC Commission Directive 92/69/EEC guidelines. These physicochemical properties determination studies were performed at Huntingdon Life Sciences Ltd., Suffolk, United Kingdom.		
Data Quality	Reliable without restrictions [Klimisch reliability 1].		
References	Unpublished confidential business information.		
Other	Date: January 7, 2004		

Acute Oral Toxicity (CAS No. 189120-64-7)

Test Substance **CAS Number** **Remarks**	Trimethylolpropane esters of heptanoic and octanoic acid 189120-64-7 100% Purity
Method/guideline	OECD 401 (1987); EC Directive (67/548/EEC) Annex V. Part B.1 (1993)
Test type **GLP** **Year**	Acute oral toxicity Yes 1999
Test system	Species: Rats (Crl:CD BR strain) Sex: Male and females. No. of animals: 10 (5 males/5 females) Weight: 223-233 grams (males) and 204-214 (females) Route: Oral gavage, undiluted test substance administered Dosage: 2000 mg/kg body weight (limit dose) Statist. Meth.: Not applicable.
Test Conditions	A group of five male and female rats (fasted overnight) were dosed orally, by stomach intubation, at a level of 2000 mg/kg of body weight. Clinical observations were performed at 1, 2, 4 and 6 hrs after dose administration and daily thereafter for a 14 day period. The animals were observed daily for a period of 14 days for mortality and signs of systemic toxicity. Body weights were recorded on days 0, 7 and 14. The animals were sacrificed and necropsied at the end of the observation period on day 14.
Results/Remarks	All animals survived treatment with test substance. Animals displayed increases in body weight over their initial values and with the exception of two males with staining of the anogenital area at the 6-hr observations, were free of observable abnormalities or overt signs of toxicity. There were no signs of macroscopic postmortem abnormalities at necropsy.
Conclusions	The acute oral LD_{50} was >2000 mg/kg for the test substance.
Data Quality	Reliable without restrictions [Klimisch reliability 1]
References	Unpublished confidential business information.
Other	Date: January 8, 2004

Repeated Dose Oral Toxicity (CAS No. 189120-64-7)

Test Substance	Trimethylolpropane esters of heptanoic and octanoic acid
CAS Number	189120-64-7
Remarks	Purity reported to be 100%
Method/guideline	OECD 407
Test type	28-Day oral toxicity study in rats
GLP	Yes
Year	2000
Species/strain	Rats /Crl:CD BR VAF/Plus, age approximately 8 weeks, weight 246 to 286 g (males), 178 to 206 g (females)
Route of Administ.	Orally by gavage
Duration of test	Twenty-eight (28) days.
No. of animals	20 males and 20 females; 5/sex/dose level
Dose/Conc. Levels	0 (carrier control), 100.0, 300.0 and 1000.0 mg/kg/day
Sex	Male and female
Frequency of treatment	Daily oral administration, 7 days/week for 4 weeks (28 days)
Control Group	Yes. Carrier (peanut oil) control group.
Statist. Methods	Bartlett's test, ANOVA, Dunnett's test, Kruskal-Wallis test, Jonckheere's test, Dunn's Summed Rank test
Post-exposure observat.	None
Remarks on Test Conditions	This study was conducted to evaluate the potential of the test substance to cause cumulative toxicity or neurotoxicity when administered orally by gavage to rats for a period of 28 days. Three groups of five male and five female rats were administered the test substance/carrier mixtures at dose levels of 100, 300, and 1000 mg/kg/day. Additionally a group of five male and five female rats served as a control and received carrier (peanut oil). Dosing volume levels were adjusted weekly based on the most recent body weights. Neurotoxicity was evaluated by assessments of Functional Observational Battery (FOB) and motor activity. Clinical observations were made daily throughout the study. A complete functional observational battery was conducted on all animals prior to receiving test material and during Week 4 of dosing. Additionally, once during Weeks 1, 2, and 3, all animals were observed in a standard arena (abbreviated FOB) for changes in skin, fur, eyes, mucous membranes, occurrences of secretions, and excretions, and autonomic activity. Changes in gait, posture, and response to handling as well as the presence of tonic and clonic movements, stereotypies, or unusual behavior also were evaluated. These observations were performed using a standardized scoring system. Motor activity also was assessed using a photobeam activity system during the same intervals as the complete functional observational battery. Body weights were recorded pretest, at dose initiation (Day 0), and on Days 7, 14, 21 and 27 for all animals. Food consumption was measured weekly during the test period. Hematology, serum chemistry, and coagulation studies were performed on all animals on Day 28. A full macroscopic postmortem examination was performed on all animals and required organs were preserved. Selected organs were weighed at study termination. A range of tissues was examined microscopically. A range-finding study was conducted prior to the main study to determine the dose levels for the main study. There were no clinical signs noted during the range-finding study. Also, there were no apparent effects on body weights and food consumption. Based on these results the high dose for the main study was set at the limit dose of 1000 mg/kg/day. The mid- and low-doses were set at approximately half-log intervals from the high dose.

Results	There were no statistically significant differences observed for the functional observational battery parameters or motor activity. All animals displayed increases in body weight over their initial values. There were no biologically significant differences in mean body weight or mean food consumption between treated and control animals at any interval. There were several statistically significant differences from control in the clinical pathology parameters. However, the only change noted at the microscopic examination was an increase in the number of hyaline droplets in the proximal cortical tubular epithelium of the 300 and 1000 mg/kg males. No signs of hyaline droplet nephropathy or renal cast were detected in these kidneys. There were no corresponding microscopic changes in the other tissues. Therefore, with the exception of the observed hyaline droplets, all other differences in clinical pathological parameters were not considered biologically significant. In conclusion, oral administration of the test substance to rats by gavage did not produce signs of overt systemic toxicity at any dose level tested. There were no treatment-related clinical inlife, functional observation battery, or gross postmortem findings. There was no treatment-related mortality; and no adverse effects on body weight, food consumption, clinical laboratory parameters, or organ weights. However, there were increased numbers of hyaline droplets in the proximal cortical tubular epithelium of the 300 and 1000 mg/kg males. Therefore, a No Observable Adverse Effect Level (NOAEL) for the test substance was established at 100 mg/kg/day.
Conclusions	NOAEL was 100 mg/kg b.w. based on no evidence of microscopic changes in histopathological examination.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: January 16, 2004

Genetic Toxicity in Vitro (CAS No. 189120-64-7)

Test Substance **CAS Number** **Remarks**	Trimethylolpropane esters of heptanoic and octanoic acid 189120-64-7 100% Purity .
Method/guideline **Type of Study** **Test System** **GLP** **Year**	OECD 471 (1997); EC 67/548/EEC Annex V. Part B.14 (1993) Bacterial Reverse Mutation Assay Bacterial (*Salmonella typhimurium*) Yes 2000
Species/Strain **Metab. Activation** **Concentrations** **Statist. Methods**	*Salmonella typhimurium* / TA98, TA100, TA102, TA1535, TA1537 Aroclor 1254-induced rat liver preparations (S9 mixture) Range finding concentrations: 50, 158, 500, 1580 and 5000 µg/plate Definitive study concentrations: 62.5, 125, 250, 500 and 1000 µg/plate. Mean revertant colony count and std deviation (Snedor and Cochran, 1989)
Test Conditions/ Remarks	Negative control: acetone or DMSO (vehicle) Positive controls: 2AA (all strains with S9 except TA102), DAN (TA102 with S9), 2NF (TA98 without S9), MNNG (TA100, TA1535 without S9), 9AA (TA1537 without S9), MMC (TA102 without S9). Abbrev. 2AA (2-Aminoanthracene); DAN (Danthron); 2NF (2-Nitrofluorene); MNNG (N-Methyl-N-Nitro-N-Nitrosoguanidine); 9AA (9-Aminoacridine); MMC (Mitomycin C) Procedure: There were 3 plates /dose groups/treatment. Samples of bacteria (0.1 mL), followed by vehicle (100 µL), appropriate test substance dilution (100 µL) or appropriate positive control substance dilution (100 µL), and 0.5 mL of S9 mix (+S9) or saline (-S9), were added to sterile glass test tubes containing molten top agar. The mixture was vortexed and immediately poured on plates containing a layer of minimal agar medium. After the top agar solidified the plates were inverted and incubated at 37 C for approximately 2 days. All plates were evaluated after approximately two days of incubation for gross toxic effects and total revertant colony numbers. Revertant colonies were counted via a Biotran III Colony Counter. Two positive controls and two vehicle controls were tested concurrently for each strain. The vehicle and positive controls were tested using a 100 µL sample.
Results/Remarks	The test material did not induce significant increases in mean revertant colonies (equal to or greater than two or three times the vehicle control) or toxicity in tester strains TA98, TA100, TA102, TA1535, or TA1537 at any dose level tested with or without metabolic activation in either the initial or repeat assays. Beading of the test substance (a common finding with materials having low water solubility) was observed in all tester strains with and without metabolic activation on all plates ≥ 1580 µg/plate in the initial assay. Beading of test substance was observed in all tester strains (except TA1537 (+S9)) with and without metabolic activation at 1000 µg/plate in the repeat assay. The test substance was negative for mutagenic activity in the five *Salmonella* tester strains, with or without metabolic activation. No mutagenic activity was observed at concentrations tested. The positive controls gave the appropriate responses as expected.
Conclusions	The test substance was <u>not</u> mutagenic, with or without metabolic activation in the *Salmonella*/Mammalian Microsome Reverse Mutation assay.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: January 8, 2004.

Genetic Toxicity In Vitro (CAS No. 189120-64-7)

Test Substance	Trimethylolpropane esters of heptanoic and octanoic acid
CAS Number	189120-64-7
Remarks	100% Purity
Method/guideline	OECD 473 (1997); 67/548/EEC, Annex V, Part B.10 (1993)
Type of Study	Chinese hamster ovary (CHO) cell assay , in vitro mammalian cytogenetic test
Test System	Mammalian cell
GLP	Yes
Year	2000
Species/Strain	Chinese Hamster Ovary (CHO) cells.
Metab. Activation	With and without Arochlor-induced rat liver S9 mixture.
Concentrations	25, 79, 250, 791, and 2500 μg/mL. These concentrations were selected based on the results of a toxicity pretest. The test substance was dissolved in acetone
Control Groups	Positive Controls were either 9,10-Dimethyl-1,2-benzanthracene [DMBA], or 1-Methyl-3-Nitro-1-Nitrosoguanidine [MNNG]). The concurrent negative control was the vehicle (acetone).
Statist. Methods	Fisher Exact Probability test, Hoeffding permutation test for dose-related trends
Test Conditions/ Remarks	The definitive study consisted of two phases: an initial chromosomal aberration assay with a 19 hour harvest time, and a repeat assay with both 19 and 43 hour cell harvest times. The CHO cells were cultured in McCoy's 5A Medium containing 10% fetal bovine serum and 2 mM L-glutamine at 37±2°C, in 4-6% CO_2 in air. Two sets of duplicate cultures were prepared; one set was treated with the test substance with activation and the other was treated with the test substance without activation. Each flask received a 50 μL sample of the test substance mixture, positive control, DMBA or MNNG, or appropriate vehicle (acetone). Flasks were treated for 3 hours for both +S9 and -S9 in the initial assay and 3 hours for +S9 and 19 hours for -S9 in the repeat assay. The cultures were incubated to their respective harvest times (19 or 43 hours). A spindle inhibitor was added to the flasks approximately 2-3 hours prior to harvest to arrest the cells in c-metaphase. The cells were harvested and slides prepared to evaluate chromosomes. The positive control materials were evaluated for chromosomal aberrations at the 19 hour harvests only.
Results/Remarks	The percentage of aberrant cells for the test substance did not exceed 5% for any of the treatment groups, even at the highest test concentration (2500 μg/m). Statistically significant differences were not observed between the treated and vehicle control groups in the percentage of aberrant cells following treatment with the test substance, with or without metabolic activation, for either the initial or repeat assays. Therefore, the test material did not induce chromosomal aberrations in this in vitro mammalian cytogenetic test system. The positive controls (i.e., DMBA and MNNG) and vehicle controls gave the appropriate response as expected.
Conclusions	The test material did not induce chromosomal aberrations in CHO cells.
Data Quality	Reliable without restrictions [Klimisch reliability 1]
References	Unpublished confidential business information.
Other	Date: January 9, 2004

Genetic Toxicity In Vivo (CAS No. 189120-64-7)

Test Substance	Trimethylolpropane esters of heptanoic and octanoic acid
CAS Number	189120-64-7
Remarks	100% Purity
Method/guideline	OECD 474 (1997)
Type of Study	*In vivo* micronucleus assay
Test system	Bone marrow and peripheral blood cells
GLP	Yes
Year	2000
Species/Strain	Mouse / CRL:CD-1(ICR)BR, 8-weeks old
Sex	Male
No. of animals	5 male/dose for three doses, vehicle (corn oil) control and positive control (
Route of Administ.	Oral gavage (diluted in corn oil vehicle)
Doses/conc. levels	500, 1000 and 2000 mg/kg
Exposure period	Two single oral doses administered approx. 24 hrs apart.
Controls	Vehicle control and positive control (cyclophosphamide, 20 mg/kg)
Statist. Methods	ANOVA, Duncan's multiple range test, Wilk's criterion or the Kolomogorov-Smirnov statistics test, Kruskal-Wallis one-way ANOVA, Dunn's summed rank test, Jonkheere's test of ordered response.
Test Conditions/	Prior to the start of the assay, a range finding study was performed. Based on the results of the range finding study, the test substance was administered via oral gavage to three groups of 5 male mice at doses of 500, 1000, and 2000 mg/kg. A fourth group of mice served as a carrier control and received corn oil only. A fifth group served as a positive control and received 20 mg/kg of cyclophosphamide via oral gavage. The test substance/carrier mixtures, carrier, and positive control substance mixtures were administered in two treatments, approximately 24 hours apart. Clinical observations were made after each test substance administration and prior to terminal sacrifice. Body weights were recorded before testing, on the first day of dosing, and on the day of death. All animals were sacrificed approximately 24 hours following the last test substance administration. Immediately after sacrifice, both femurs were removed from each animal and processed. Bone marrow smears were prepared, 2 slides per animal, and stained using acridine orange. Two thousand polychromatic erythrocytes (PCEs) from each animal were examined for the presence of micronuclei. The percentage of PCEs was determined by evaluation of the first 1000 erythrocytes counted. All animals survived to scheduled study termination. All animals in all groups were within normal limits for the entire study with the exception of one control animal and one 2000 mg/kg group animal that had little sign of stool on the day of sacrifice.
Remarks	This study was conducted in order to evaluate the potential of the test substance to induce micronucleated polychromatic erythrocytes (MNE) in the bone marrow in CD-1 mice. The *in vivo* mammalian bone marrow micronucleus assay is a short term test to evaluate the clastogenic (chromosome breaking) potential of test materials. Evidence of chromosome breakage or nondisjunction can be readily detected as MNEs.
Results	There were no dose-related increases or statistically significant differences in micronuclei formation at any dose level of the test material evaluated in the mice. There was a statistically significant increase in the mean% number of polychromatic erythrocytes (PCEs) in the 2000 mg/kg animals. No evidence of cytotoxicity was observed during the study. The positive controls (cyclophosphamide) induced a statistically significant increase in the mean number of MNE/2000 PCE compared with controls, indicating that the test system responded in an appropriate manner.

Conclusions	The test material did not produce any increase in micronuclei formation in PCEs at any of the dose levels. Hence, this test material did not cause chromosome damage in this test.
Data Quality	Reliable without restrictions [Klimisch reliability 1]
References	Unpublished confidential business data.
Other	Date: January 12, 2004

Acute fish toxicity (CAS No. 189120-64-7)

Test Substance	Trimethylolpropane esters of heptanoic and octanoic acid
CAS Number	189120-64-7
Remarks	100% Purity
Method/guideline	OECD 203 (1992), 67/548/EEC, Annex V, Part C.1 (1993)
Type (test type)	Acute fish toxicity study
Test System	Fish, freshwater
GLP	Yes
Year	1999
Species/Strain	Fish: Rainbow trout (*Oncorhynchus mykiss)*
Analyt. Monitoring	Analyses of WAF solutions were performed by GC-FID
Exposure period	96 hours
Statist. Methods	Not indicated
Test Conditions	96-hr semi-static (renewal) acute fish toxicity test was carried out with water accommodated fractions (WAFs) of the test material at five nominal concentrations ranging from 62.5 mg/L to 1000 mg/L Species: Rainbow trout (*Oncorhynchus mykiss*), mean length 43 ± 3 mm Test performed in 8.5 L glass aquaria containers vessels containing 5.0 L of the WAF solutions prepared from laboratory dilution water (hardness 150 mg/L $CaCO_3$); 14.1-15.5°C; 16 h light/8h dark cycle; unfed; mean loading 0.59 g/L. The water accommodated fractions (WAFs) were prepared in 13-L aspirator using 12L of laboratory dilution water and the appropriate amounts of the test material to achieve the nominal concentrations desired. The WAF mixtures were stirred at room temperature for approx 24 hrs and allowed to settle for approx. 1 hr before the WAF solutions were removed through an outlet at the bottom of the 13L aspirator container. No. of fish: 10/treatment WAF Concentrations (nominal): 0 (untreated controls), 62.5, 125, 250, 500 and 1000 mg/L Physical Measurement: The pH, temperature and dissolved oxygen were performed daily. During course of 96 hr study, the pH in WAF solutions varied by more than one unit but was considered acceptable since no mortality was observed during the 96-hr period. Dissolved oxygen levels remained above 60% saturation for all treatment and temperature ranged from 14.1 to 15.5 °C. WAF solutions were taken on 0, 24, 48, 72 and 96 hrs and analyzed by GC-FID for the test material. GC-FID limit of detection for test material was 0.184 mg/L. Observations: Mortality/symptoms at 3, 24, 48, 72 and 96 hr
Results/Remarks	WAF Nominal conc. Loading Level (mg/L) / Mean Measured Concentration (mg/L) / Mortality (96-hr) 0 / not detected / 0% 62.5 / 0.07 / 0 125 / 0.13 / 0 250 / 0.14 / 0 500 / 0.21 / 0 1000 / 0.42 / 0

	No mortality was observed in the fish at any of the WAF solutions during the 96-hr exposure period. GC-FID analyses of WAF solutions indicated that test material was present in the range of 0.07 to 0.42 mg/L. Hence, it appears that test material has limited solubility in the WAF solution tested and this is consistent with previous water solubility determination (less than 0.1 mg/L, measured) of this test substance.
Conclusion	The 96-hr LC_{50} or 96-hr LL_{50} was >1000 mg/L WAF (nominal concentration) in which the measured water concentration was 0.42 mg/L (GC-FID). No mortality was observed at any of the tested WAF concentrations (nominal or measured). Hence, data indicate that the test material is not expected to cause mortality in fish at its maximal water solubility limit or water saturated limit (WSL).
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: January 12, 2004.

Acute toxicity to aquatic invertebrate (CAS No. 189120-64-7)

Test Substance	Trimethylolpropane esters of heptanoic and octanoic acid
CAS Number	189120-64-7
Remarks	100% Purity
Method/guideline	OECD 202 (1984), 67/548/EEC, Annex V, Part C.2 Acute Toxicity for Daphnia (1993)
Type (test type)	*Daphnia sp.* , Acute immobilization test
Test System	Freshwater invertebrate
GLP	Yes
Year	1999
Species/Strain	Freshwater invertebrate, *Daphnia magna*
Analyt. Monitoring	Analyses of WAF solutions were performed by GC-FID
Exposure period	48 hours
Statist. Methods	Not indicated
Test Conditions	48-hr static acute immobilization study was carried out with water accommodated fractions (WAFs) of the test material at five nominal concentrations ranging from 62.5 mg/L to 1000 mg/L Species *Daphnia magna*, <24 h old Test was performed at ca. 20°C in 125 mL glass beakers containing sufficient volume (so that there is no headspace) of the water accommodated fraction (WAF) solutions prepared from laboratory dilution water (hardness 150 mg/L $CaCO_3$); 16 h light/8h dark cycle; daylight intensity 675 lux; unfed.; loading > 2 mL solution per daphnid The WAF solutions were prepared in glass aspirator bottles using 12L of laboratory dilution water and the appropriate amounts of the test material to achieve the nominal concentrations desired. The WAF mixtures were stirred (<10% vortex) at room temperature for approx 24 hrs and allowed to settle for approx. 1 hr before the WAF solutions were removed through an outlet at the bottom of the aspirator bottle. No. of daphnids: 10 /replicate, 2 replicates/treatment WAF Concentrations (nominal): 0 (untreated controls), 62.5, 125, 250, 500 and 1000 mg/L Physical Measurement: The pH, temperature and dissolved oxygen measurements were performed on Day 0 and 2. During course of 48-hr study, the pH in WAF solutions ranged from 7.8 to 8.3; dissolved oxygen levels remained clearly above 60% saturation for all treatment (range 8.0-8.2 mg O_2/L), and temperature ranged from 19.6-20.0 °C. WAF solutions were taken at 0 and 48 hrs and analyzed by GC-FID for the test material. GC-FID

	limit of detection for test material was 0.184 mg/L. Observations: Immobilization and symptoms were observed daily.
Results/Remarks	WAF Nominal conc. Loading Level (mg/L) / Mean Measured Concentration (mg/L) / % Immobility (48-hr) 0 / not detected / 0% 62.5 / 0.09 / 0 125 / 0.17 / 0 250 / 0.20 / 0 500 / 0.34 / 0 1000 / 0.59 / 0 No immobilization was observed in the daphnids at any of the WAF solutions during the 48-hr exposure period. GC-FID analyses of WAF solutions indicated that test material was present in the range of 0.09 to 0.59 mg/L. Hence, it appears that test material has limited solubility in the WAF solution tested and this is consistent with previous water solubility determination (less than 0.1 mg/L, measured).
Conclusion	The 48-hr EC_{50} or 48-hr EL_{50} was >1000 mg/L WAF (nominal concentration) in which the measured water concentration was 0.59 mg/L (GC-FID). No immobilization or abnormal behavior was observed with any of the tested WAF solutions. Hence, data indicate that the test material is not expected to cause immobilization in daphnids at or close to its maximal water solubility limit or water saturated limit (WSL).
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: January 12, 2004.

Acute toxicity to aquatic plants (e.g., algae) (CAS No. 189120-64-7)

Test Substance	Trimethylolpropane esters of heptanoic and octanoic acid
CAS Number	189120-64-7
Remarks	100% Purity
Method/guideline	67/548/EEC, Annex V, Part C.3 Algal inhibition test (1993)
Type (test type)	Algae, growth inhibition study
Test System	Aquatic plant (e.g., algae)
GLP	Yes
Year	1999
Species/Strain	Green algae / *Selenastrum capricornutm*
Analyt. Monitoring	Analyses of WAF solutions were performed by GC-FID
Exposure period	72 hours
Statist. Methods	ANOVA, SAS regression analysis
Test Conditions	72-hr static algae growth inhibition study was carried out with water accommodated fractions (WAFs) of the test material at five nominal concentrations ranging from 62.5 mg/L to 1000 mg/L. Species: Green algae (*Selenastrum capricornutum*) Tests were performed in 125 mL flasks containing 60 mL of WAF-algal medium solutions (pH 7.4-7.6); temperature: 23 ± 1°C; continuous illumination (~4300 lux); continuously shaken at 100 rpm. Sufficient alga was added to obtain the initial cell count for the experiments. The WAF solutions were prepared in glass aspirator bottles using 4L of algal algal nutrient

	medium solution and the appropriate amounts of the test material to achieve the nominal concentrations desired. The solution mixtures were stirred (<10% vortex) at room temperature for approx 24 hrs and allowed to settle for approx. 1 hr before the WAF solutions were removed through an outlet at the bottom of the aspirator bottle. Initial Cell Conc.: 1 x 10^4 cells/mL No. of replicates: 4/treatment WAF Concentrations (nominal): 0 (untreated controls), 62.5, 125, 250, 500 and 1000 mg/L Physical Measurements: pH was determined at 0 and at 72 hrs (termination). Observations: Cell density was determined for each replicate at 24, 48 and 72 hr by using a hemacytometer. WAF solutions were taken at 0 and 72 hrs and analyzed by GC-FID for the test material. GC-FID limit of detection for test material was 0.184 mg/L.
Results/Remarks	WAF Nominal conc. / Loading Level (mg/L)	Mean Measured Concentration (mg/L)	% Inhibition (0-72h) (relative to controls) Growth Rate	AUC-Growth Curve
	62.5	<0.04	6.9%	2.0%
	125	0.07	10.2	16.1
	250	0.08	9.0	- 0.5
	500	0.09	5.8	4.5
	1000	0.23	25.3	37.5
	Algal inhibition was not significantly apparent except at the highest nominal WAF concentration of 1000 mg/L [which showed a 25.3% inhibition (based on growth rate) and 37.5% inhibition (based on area under the curve-growth rate)]. The 72-hr NOEC was considered to be 500 mg/L WAF (nominal conc.). GC-FID analyses of WAF solutions indicated that test material was present in the range of <0.04 to 0.23 mg/L. Hence, it appears that test material has limited solubility in the WAF solution tested and this is consistent with previous water solubility determination (less than 0.1 mg/L, measured).
Conclusion	The 72-hr EC_{50} or 72-hr EL_{50} was expected to be >1000 mg/L WAF (nominal concentration). The 72-hr NOEC was 500 mg/L WAF (measured 0.09 mg/L). Hence, data indicate that the test material is not expected to cause inhibition to algae at or close to its maximal water solubility limit or water saturated limit (WSL).
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: January 13, 2004.

Biodegradation (CAS No. 189120-64-7)

Test Substance	Trimethylolpropane esters of heptanoic and octanoic acid
CAS Number	189120-64-7
Remarks	100% Purity
Method/guideline	OECD Guideline 301F (1993), Ready Biodegradability: Manometric Respirometry Test.
Test type	Aerobic Biodegradation
GLP	Yes
Year	2000
Test system	Exposure Period: 28 Days Inoculum: Activated Sludge, Domestic Bacterial population was 1 x 10^7 CFU/ml Kinetics: Not Reported Biodegradation Products: Not Reported

	Analytical Monitoring: Oxygen uptake monitored
Test Conditions	Treatment replicates were prepared by combining glass-distilled water, a mineral substrate, pH buffer, activated sludge and the appropriate test substance. Three replicates of the test material and three replicates of positive control (sodium benzoate) were prepared and evaluated in 1L glass vessels. Three blank controls were also used to background subtract Oxygen consumed by microorganisms from the oxidation of the test substance was continuously monitored using an automated respirometer. Test flasks were continuously stirred for 28 days in the dark. Test temperature was 22 ± 1 °C. The pH was 7.04-7.26 at the end of the 28-day study. Concentrations for Test Substance was 50.43 mg/L for test substance. Concentration for Sodium Benzoate (positive control) was 48.54 mg/L
Results	Biodegradation was 68.56% in 28 days (n=3) for the test material. Data indicated that the test material was not readily biodegradable (did not meet "10-day window" criterion). Sodium benzoate, the positive control reference substance, biodegraded to the extent of 90.38% in 28 days and met the "10-day window" criterion for "readily biodegradable" classification. The biodegradation calculation was performed using the respirometry software from the instrument's manufacturer [Co-ordinated Environmental Service (Kent, UK)], the Theoretical Oxygen Demand (ThOD) and the amount of the test substance added. ThOD of the test material was 2.49 and was based upon the elemental analysis of the test substance (69.39% Carbon, 10.64% Hydrogen, and 20.14% Oxygen).
Conclusions	The test substance was not readily biodegradable.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information
Other	Date: January 13, 2004

Boiling Point, Vapor Pressure, Partition Coefficient, and Water Solubility (CAS No. 180788-27-6) Hexanedioic acid, mixed esters with C10-rich, C9-C11 alcohols and TMP-Surrogate Polyol Ester

Test Substance **CAS Number** **Remarks**	**Hexanedioic acid, mixed esters with C10-rich, C9-C11 alcohols and TMP** **180788-27-6** **Purity was 100%**		
	GLP (Yes/No)	**METHOD/ GUIDELINE**	**RESULTS / CONCLUSIONS**
Physicochemical Properties			
Boiling Point	Yes	OECD 103	> 250 °C (not determinable, decomposes at temp above 250°C without boiling)
Vapor Pressure	Yes	OECD 104	1.7×10^{-5} Pascals at 25 °C
Partition Coeffic.	Yes	OECD 107	log P > 6 (estimated from n-octanol solubility and water solubility of test material)
Water Solubility	Yes	OECD 105	0.41 mg/L (GC analysis)
Year	1996		
Remarks	Determination of a complete battery of physicochemical properties for the test substance CAS No. 180788-27-67, including those designated above has been carried out under GLP and by methods, which are in compliance with the OECD and EEC Commission Directive 92/69/EEC guidelines. These physicochemical properties determination studies were performed at Huntingdon Life Sciences Ltd., Suffolk, United Kingdom.		
Data Quality	Reliable without restrictions [Klimisch reliability 1].		
References	Unpublished confidential business information.		
Other	Date: January 9, 2004		

Acute Oral Toxicity (CAS No. 180788-27-6)

Test Substance	Hexanedioic acid, mixed esters with C10-rich, C9-C11 alcohols and TMP
CAS Number	180788-27-6
Remarks	100% Purity
Method/guideline	OECD 401 (1987); EC Directive (67/548/EEC) Annex V. Part B.1 (1993)
Test type	Acute oral toxicity
GLP	Yes
Year	1996
Test system	Species: Rats (Crl:CD BR strain), 8-10 weeks old Sex: Male and females. No. of animals: 10 (5 males/5 females) Weight: 229-243 gm (males) and 170-194 gm (females) Route: Oral gavage, undiluted test substance administered Dosage: 2000 mg/kg body weight (limit dose) Statist. Meth.: Not applicable.
Test Conditions	A group of five male and female rats (fasted overnight) were dosed orally, by stomach intubation, at a level of 2000 mg/kg of body weight. Clinical observations were performed at 1, 2, 4 and 6 hrs after dose administration and daily thereafter over a period of 14 days. The animals were observed daily for a period of 14 days for mortality and signs of systemic toxicity. Body weights were recorded on days 0, 7 and 14. The animals were sacrificed and necropsied at the end of the observation period on day 14.
Results/Remarks	All animals survived treatment with test substance and gained weight over their initial (Day 0) values. Clinical observations were reported on Day 0 which include one male with soft stool and two females with anogenital staining. Oral dose of test material did not produce any consistent signs of systemic toxicity and all animals were free of abnormalities on Day 1 through Day study termination on Day 14 and at postmortem necropsy examination.
Conclusions	The acute oral LD_{50} was >2000 mg/kg for the test substance.
Data Quality	Reliable without restrictions [Klimisch reliability 1]
References	Unpublished confidential business information.
Other	Date: January 12, 2004

Repeated Dose Toxicity (CAS No. 180788-27-6)

Test Substance	Hexanedioic acid, mixed esters with C10-rich, C9-C11 alcohols and TMP
CAS Number	180788-27-6
Remarks	100% Purity
Method/guideline	OECD 407(1981); 67/548/EEC. Annex V, Part B.7 (1993)
Test type	28-Day Oral Toxicity in Rats
GLP	Yes
Year	1996
Species/strain	Rats/Crl:CD BR, age 7-8 weeks, weight 190-211 (males), 157-186 (females)
Route of Administration	Oral gavage (in corn oil carrier vehicle)
Duration of test	4-weeks
No. of animals	Four groups of 5 males and 5 females

	Group 1 (control, corn oil vehicle); Group 2 (100 mg/kg); Group 3 (300 mg/kg); Group 4 (1000 mg/kg)
Dose/Conc. Levels	0.0, 100, 300 or 1000 mg/kg/day
Sex	Males and females
Frequency of treatment	Single oral gavage /day, 7 days/week for 4 weeks.
Control Group	Yes. 5 male and 5 female (administrated corn oil only)
Statist. Methods	Analysis of variance (ANOVA), associated F-test, Dunnett's test, Bartlett's test, Kruskal-Wallis test, Dunn's summed rank test, Jonckheeree's test for dose-response trends.
Post-exposure observat.	Mortality, survival, growth, food consumption, clinical signs/symptoms, clinical chemistry, hematology, necropsy, gross morphology and histopathology were carried out . Body weights were recorded on Day 0, 7, 14, 21, 27 and on day of sacrifice.
Remarks on Test Conditions	Blood samples were collected on Day 28 and hematology, serum chemistry, clotting potential analyses were performed. All animals were subjected to gross necropsy. A full macroscopic postmortem examination performed on all animals and required organs were preserved. Organs were weighed and preserved including the following: liver, kidneys, testes/ovaries, epididymides, uterus, brain, adrenals. Histopathology was carried out for the control and high dose group animals. Tissues examined were liver, kidneys, spleen, adrenals, heart, lungs, testes/ovaries, gross lesions; they were processed, sectioned, stained (hematoxylin and eosin) and examined microscopically.
Results	All animals survived to scheduled study termination and were free of clinical signs of toxicity throughout the test period. There were no biological significant differences in mean body weight, food consumption, hematology, clotting potential, serum chemistry parameters or absolute organ weights between treated and control animals at any interval. There were no postmortem findings that were considered related to the treatment with the test material. Microscopic changes observed in the kidneys of all treated males (100, 300 and 1000 mg/kg) consisted of the presence of hyaline droplets in the cortical tubular epithelium. This effect is characteristic of kidney nephropathy in male rats which is associated with many chemicals. Consistent with EPA guidance, these findings were not considered in the estimation of the NOAEL for the test substance. There were adaptive changes seen in the liver of the 1000 mg/kg dose males and females which consisted of an increase in relative liver weight and enlarged hepatocytes. The increase in relative liver weight could possibly be attributed to a normal physiological adaptive response by the liver to metabolize an exogenous agent rather than a true toxic event. Thus, in the absence of any other histopathological findings or clinical pathology, these adaptive changes were not considered to be toxicologically significant. Overall, the repeated oral administration of the test substance to rats did not produce chemical specific signs of systemic toxicity at any dose level tested. The kidney hyaline droplet formation in male rats only and the adaptive changes in the liver were not considered toxicological significant and were not considered in the estimation of the NOAEL.
Conclusions	NOAEL was established at 1000 mg/kg/day in both male and female rats under the conditions of this study. There were no treatment-related clinical in-life or gross postmortem findings, no treatment-related mortality, no adverse effects on body weight, food consumption, clinical laboratory parameters in either male or female animals.
Data Quality	Reliable without restrictions [Klimisch reliability 1]
References	Unpublished confidential business information.
Other	Date: January 16, 2004

Genetic Toxicity in Vitro (CAS No. 180788-27-6)

Test Substance	Hexanedioic acid, mixed esters with C10-rich, C9-C11 alcohols and TMP
CAS Number	180788-27-6
Remarks	100% Purity.
Method/guideline	OECD 471 (1983); EC 67/548/EEC Annex V. Part B.14 (1993)
Type of Study	Bacterial Reverse Mutation Assay
Test System	Bacterial (*Salmonella typhimurium*)
GLP	Yes
Year	1997
Species/Strain	*Salmonella typhimurium* / TA98, TA100, TA1535, TA1537 , TA 1538
Metab. Activation	Aroclor 1254-induced rat liver preparations (S9 mixture)
Concentrations	Test concentrations: 50, 100, 500, 1000 and 5000 µg/plate
Statist. Methods	Mean revertant colony count and std deviation (Snedor and Cochran, 1989)
Test Conditions/ Remarks	Negative control: acetone or DMSO (vehicle) Positive controls: 2AA (all strains with S9), 2NF (TA98, TA 1538 without S9), MNNG (TA100, TA1535 without S9), 9AA (TA1537 without S9). Abbrev. 2AA (2-Aminoanthracene); 2NF (2-Nitrofluorene); MNNG (N-Methyl-N-Nitro-N-Nitrosoguanidine); 9AA (9-Aminoacridine); Procedure: There were 3 plates /dose groups/treatment. Samples of bacteria (0.1 mL), followed by vehicle (acetone or DMSO) (100 µL), appropriate test substance dilution (100 µL) or appropriate positive control substance dilution (100 µL), and 0.5 mL of S9 mix (+S9) or saline (-S9), were added to sterile glass test tubes containing molten top agar. The mixture was vortexed and immediately poured on plates containing a layer of minimal agar medium. After the top agar solidified the plates were inverted and incubated at 37 ± 2 °C for approximately 2 days. All plates were evaluated after approximately two days of incubation for gross toxic effects and total revertant colony numbers. Revertant colonies were counted via a Biotran III Colony Counter. Two positive controls and two vehicle controls were tested concurrently for each strain. The vehicle and positive controls were tested using a 100 µL sample.
Results/Remarks	The test material did not induce significant increases in mean revertant colonies (equal to or greater than two or three times the vehicle control) or toxicity in tester strains TA98, TA100, TA1535, TA1537 or TA 1538 at any dose level tested with or without metabolic activation in assays. The test substance was negative for mutagenic activity in the five *Salmonella* tester strains, with or without metabolic activation. No mutagenic activity was observed at concentrations tested. The positive controls gave the appropriate responses as expected.
Conclusions	The test substance was <u>not</u> mutagenic, with or without metabolic activation in the *Salmonella*/Mammalian Microsome Reverse Mutation assay.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: January 12, 2004.

Genetic Toxicity in Vitro (CAS No. 180788-27-6)

Test Substance **CAS Number** **Remarks**	Hexanedioic acid, mixed esters with C10-rich, C9-C11 alcohols and TMP 180788-27-6 100% Purity.
Method/guideline	67/548/EEC, Annex V, Part B.10 (1993)
Type of Study **Test System** **GLP** **Year**	Chinese hamster ovary (CHO) cell assay , in vitro mammalian cytogenetic test Mammalian cell Yes 1997
Species/Strain **Metab. Activation** **Concentrations** **Control Groups**	Chinese Hamster Ovary (CHO) cells. With and without Arochlor-induced rat liver S9 mixture. 10, 20, 39, 78, 156, 313, 625, 1250 and 2500 μg/mL (initial assay) and 625, 1250 and 2500 μg/mL (repeat assay). These concentrations were selected based on the results of a toxicity pretest. The test substance was dissolved in acetone Positive Controls were either 9,10-Dimethyl-1,2-benzanthracene [DMBA](+S9), or 1-Methyl-3-Nitro-1-Nitrosoguanidine [MNNG] (-S9). The concurrent negative control was the vehicle (acetone).
Statist. Methods	Fisher Exact Probability test, Hoeffding permutation test for dose-related trends
Test Conditions/ Remarks	The definitive study consisted of two phases: an initial chromosomal aberration assay with a 20 hour harvest time, and a repeat assay with both 20 and 44 hour cell harvest times. The CHO cells were cultured in McCoy's 5A Medium containing 10% fetal bovine serum and 2 mM L-glutamine at 37±2°C, in 4-6% CO_2 in air. Two sets of duplicate cultures were prepared; one set was treated with the test substance with activation and the other was treated with the test substance without activation. Each flask received a 50 μL sample of the test substance mixture, positive control mixture, DMBA or MNNG, or vehicle (acetone). Flasks with (+S9) and without (-S9) metabolic activation were treated for 3 and 20 hrs, respectively. The cultures were incubated to their respective harvest times (20 or 44 hours). A spindle inhibitor was added to the flasks approximately 2-hours prior to harvest to arrest the cells in c-metaphase. The cells were harvested and slides prepared to evaluate chromosomes. The positive control materials were evaluated for chromosomal aberrations at the 20 hour harvests only.
Results/Remarks	No statistically significant differences were observed in the percentage of aberrant cells following treatment with the test material, either with or without metabolic activation for the initial or repeat assays. Furthermore, there were no apparent dose-related trends which would indicate a relationship to treatment. The positive controls (i.e., DMBA and MNNG) and vehicle controls gave the appropriate responses as expected.
Conclusions	The test material did not induce chromosomal aberrations in CHO cells.
Data Quality	Reliable without restrictions [Klimisch reliability 1]
References	Unpublished confidential business information.
Other	Date: January 12, 2004

Genetic Toxicity In Vivo (CAS No. 180788-27-6)

Test Substance	Hexanedioic acid, mixed esters with C10-rich, C9-C11 alcohols and TMP
CAS Number	180788-27-6
Remarks	100% Purity.
Method/guideline	OECD 474 (1983), 67/548/EEC, Annex V, Part B.12 (1993)
Type of Study	*In vivo* micronucleus assay
Test system	Bone marrow and peripheral blood cells
GLP	Yes
Year	1996
Species/Strain	Mouse / CD-1, approx 9 weeks old
Sex	25 male and 25 female
No. of animals	5 animals/dose for three doses, vehicle (corn oil) control and positive control (cyclophosphamide, 20 mg/kg)
Route of Administ.	Oral gavage (diluted in corn oil vehicle)
Doses/conc. levels	500, 1000 and 2000 mg/kg
Exposure period	Two single oral doses administered approx. 24 hrs apart.
Controls	Vehicle control and positive control (cyclophosphamide, 20 mg/kg)
Statist. Methods	ANOVA, Duncan's multiple range test, Wilk's criterion or the Kolomogorov-Smirnov statistics test, Kruskal-Wallis one-way ANOVA, Dunn's summed rank test, Jonkheere's test of ordered response.
Test Conditions/	Prior to the start of the assay, a range-finding study was performed. Based on the results of the range finding study, the test substance was administered via oral gavage to three groups of 5 male and 5 female mice at doses of 500, 1000, and 2000 mg/kg. A fourth group of mice served as a carrier control and received corn oil only. A fifth group served as a positive control and received 20 mg/kg of cyclophosphamide via oral gavage. The test substance/carrier mixtures, carrier, and positive control substance mixtures were administered in two treatments, approximately 24 hours apart. Clinical observations were made after each test substance administration and prior to terminal sacrifice. Body weights were recorded before testing, on the first day of dosing, and on the day of death. All animals were sacrificed approximately 24 hours following the last test substance administration. Immediately after sacrifice, both femurs were removed from each animal and processed. Bone marrow smears were prepared, 2 slides per animal, and stained using acridine orange. Two thousand polychromatic erythrocytes (PCEs) from each animal were examined for the presence of micronuclei. The percentage of PCEs in the total population of erythrocytes was determined by counting 1000 PCEs and normochromatic erythrocytes (NCEs). All animals survived to scheduled study termination and were free of treatment-related abnormalities for the study.
Remarks	This study was conducted in order to evaluate the potential of the test substance to induce micronucleated polychromatic erythrocytes (MNE) in the bone marrow in CD-1 mice. The *in vivo* mammalian bone marrow micronucleus assay is a short-term test to evaluate the clastogenic (chromosome breaking) potential of test materials. Evidence of chromosome breakage or nondisjunction can be readily detected as MNEs.
Results	There were no dose-related increases or statistically significant differences in micronuclei formation at any dose level of the test material evaluated compared with the vehicle controls. No evidence of cytotoxicity was observed during the study. The positive controls (cyclophosphamide) induced a statistically significant increase in the mean number of MNE/2000 PCE compared with controls, indicating that the test system responded in an appropriate manner.

Conclusions	The test material did not produce any increase in micronuclei formation in PCEs at any of the dose levels. Hence, this test material did not cause chromosome damage or induce cytotoxicity in the bone marrow.
Data Quality	Reliable without restrictions [Klimisch reliability 1]
References	Unpublished confidential business data.
Other	Date: January 13, 2004

Acute fish toxicity (CAS No. 180788-27-6)

Test Substance	Hexanedioic acid, mixed esters with C10-rich, C9-C11 alcohols and TMP
CAS Number	180788-27-6
Remarks	100% Purity
Method/guideline	OECD 203 (1992), 67/548/EEC, Annex V, Part C.1 (1993)
Type (test type)	Acute fish toxicity study
Test System	Fish, freshwater
GLP	Yes
Year	1996
Species/Strain	Fish: Rainbow trout (*Oncorhynchus mykiss)*
Analyt. Monitoring	Analyses of WAF test solutions were performed by GC-FID
Exposure period	96 hours
Statist. Methods	Not indicated
Test Conditions	96-hr semi-static (renewal) acute fish toxicity test was carried out with water accommodated fractions (WAFs) of the test material at five nominal concentrations ranging from 0.13 mg/L to 2.0 mg/L. Ethanol was used as a vehicle to help solubilize the test material into solution in the preparation of the WAFs. Species: Rainbow trout (*Oncorhynchus mykiss*), mean length 39 ± 3 mm Test performed in 19L glass aquaria test chamber containing 9 L of the WAF solutions prepared from laboratory dilution water (hardness 152 mg/L $CaCO_3$); 13.0-13.9°C; 16 h light/8h dark cycle; unfed; mean loading 0.458 g/L. Preliminary experiments were carried out which showed that approx. 2.0 mg/L was the maximum achievable water-soluble conc of the test material using ethanol as vehicle. The water accommodated fractions (WAFs) were prepared in large carboy containers containing 19.5 L of laboratory dilution water and the appropriate amounts of the test material (using stock solutions of 20 mg/ml test material in ethanol) to achieve the nominal concentrations desired. The WAF mixtures were stirred at room temperature for approx 24 hrs and allowed to settle for approx. 1 hr before the WAF was siphoned out into two replicate test chambers. No. of fish: 20/treatment with 10/test chamber (duplicate) WAF Concentrations (nominal): 0 (untreated controls), 0 (ethanol vehicle control, < 0.1 ml/L)), 0.13, 0.25, 0.50, 1.0 and 2.0 mg/L Physical Measurement: The pH, temperature and dissolved oxygen were performed daily. The pH ranged from 6.9 to 7.3. Dissolved oxygen levels remained above 60% saturation for all treatment and temperature ranged from 13.0 to 13.9 °C. WAF solutions were taken on 0, 24, 72 and 96 hrs and analyzed by GC-FID for the test material. Observations: Mortality, abnormal behavior and appearance of fish at 24, 48, 72 and 96 hr

Results/Remarks	WAF Nominal conc. Loading Level (mg/L) / Mean Measured Concentration (mg/L) / Mortality (96-hr) 0 (control) / LOD / 0% 0 (vehicle control) / LOD / 0 0.13 / 0.128 / 0 0.25 / 0.351 / 0 0.5 / 0.280 / 0 1.0 / 0.565 / 0 2.0 / 1.72 / 0 LOD = limit of detection was 0.08 mg/L for GC-FID analysis No mortality was observed in the fish at any of the WAF solutions during the 96-hr exposure period. GC-FID analyses of WAF solutions indicated that test material was present in the range of 0.128 to 1.72 mg/L. Ethanol appears to help solubilize the test material into the water. The test material has a previously determined water solubility of 0.41 mg/L (no ethanol vehicle).
Conclusion	The 96-hr LC_{50} or 96-hr LL_{50} was > 2 mg/L WAF (nominal concentration) in which the measured water concentration was 1.72 mg/L (GC-FID). No mortality was observed at any of the tested WAF concentrations (nominal or measured). Hence, data indicate that the test material not expected to cause mortality in fish at or above its water solubility limit or water saturated limit (WSL).
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: January 14, 2004.

Acute toxicity to aquatic invertebrate (CAS No. 180788-27-6)

Test Substance	Hexanedioic acid, mixed esters with C10-rich, C9-C11 alcohols and TMP
CAS Number	180788-27-6
Remarks	100% Purity
Method/guideline	OECD 202 (1984), 67/548/EEC, Annex V, Part C.2 Acute Toxicity for Daphnia (1993)
Type (test type)	*Daphnia sp.* , Acute immobilization test
Test System	Freshwater invertebrate
GLP	Yes
Year	1996
Species/Strain	Freshwater invertebrate, *Daphnia magna*
Analyt. Monitoring	Analyses of WAF solutions were performed by TOC (total organic carbon)
Exposure period	48 hours
Statist. Methods	Not indicated
Test Conditions	48-hr static acute immobilization study was carried out with water accommodated fractions (WAFs) of the test material at five nominal concentrations ranging from 62.5 mg/L to 1000 mg/L Species *Daphnia magna*, <24 h old Test was performed at ca. 20°C in 125 mL glass beakers containing sufficient volume (so that there is no headspace) of the water accommodated fraction (WAF) solutions prepared from laboratory dilution water (hardness 166 mg/L $CaCO_3$); 16 h light/8h dark cycle; daylight intensity 635 lux; unfed.; loading was approximately 1 daphnid per 28 mL solution . The WAF solutions were prepared in glass aspirator bottles using 2L of laboratory dilution water and the appropriate amounts of the test material to achieve the nominal concentrations

	desired. The WAF mixtures were stirred (5 to 10% vortex) at room temperature for approx. 24 hrs and allowed to settle for approx. 1 hr before the WAF solutions were removed through an outlet at the bottom of the aspirator bottle. No. of daphnids: 20 /treatment in four replicates (5daphnids/replicate) WAF Concentrations (nominal): 0 (untreated controls), 62.5, 125, 250, 500 and 1000 mg/L Physical Measurement: The pH, temperature and dissolved oxygen measurements were performed on Day 0 and 2. During course of 48-hr study, the pH in WAF solutions ranged from 7.7 to 8.1; dissolved oxygen levels remained clearly above 60% saturation for all treatment (range 7.2-8.8 mg O_2/L), and temperature ranged from 20.8-20.0 °C. WAF solutions were taken at 0 and 48 hrs and analyzed by TOC. Observations: Immobilization and symptoms were observed daily.
Results/Remarks	WAF Nominal conc. Loading Level (mg/L) — % Immobility (48-hr) 0 — 0% 62.5 — 0 125 — 0 250 — 0 500 — 10 1000 — 5 After 48-hr exposure period, 10% immobilization was observed in the 500 mg/L nominal WAF group and 5% immobilization in the 1000 mg/L nominal WAF exposure group. No immobilization was observed in the daphnids at the control, 62.5, 125 and 250 mg/L WAF exposure groups. TOC analyses indicated that the test material was present in the WAF solution but carbon analysis concentrations were very low (Day 2 detectable values ranging from 0.40 to 1.08 mg/L).
Conclusion	The 48-hr EC_{50} or 48-hr EL_{50} was >1000 mg/L WAF (nominal concentration) in which the measured TOC was 1.08 mg/L (relative to controls). The test material has a previously determined water solubility of 0.41 mg/L. No immobilization or abnormal behavior was observed at the 62.5, 125 and 250 mg/L nominal WAF concentrations. Hence, data indicate that the test material is not expected to cause immobilization in daphnids at or close to its maximal water solubility limit or water saturated limit (WSL).
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: January 12, 2004.

Acute toxicity to aquatic plants (e.g., algae) (CAS No. 180788-27-6)

Test Substance	Hexanedioic acid, mixed esters with C10-rich, C9-C11 alcohols and TMP
CAS Number	180788-27-6
Remarks	100% Purity
Method/guideline	OECD 201 (1984); 67/548/EEC, Annex V, Part C.3 Algal inhibition test (1993)
Type (test type)	Algae, growth inhibition study
Test System	Aquatic plant (e.g., algae)
GLP	Yes
Year	1996
Species/Strain	Green algae / *Selenastrum capricornutm*
Analyt. Monitoring	Analyses of WAF solutions were carried out using TOC analysis

Exposure period	72 hours
Statist. Methods	ANOVA, SAS regression analysis
Test Conditions	72-hr static algae growth inhibition study was carried out with water accommodated fractions (WAFs) of the test material at five nominal concentrations ranging from 62.5 mg/L to 1000 mg/L. Species: Green algae (*Selenastrum capricornutum*) Tests were performed in 125 mL flasks containing approx. 50 mL of WAF-algal medium solutions (pH 7.3-7.5); temperature: 21.8 ± 0.2°C; continuous illumination (~4400-4500 lux); continuously shaken at 100 rpm. Sufficient alga was added to obtain the initial cell count for the experiments. The WAF solutions were prepared in glass aspirator bottles using 2L of algal nutrient medium solution and the appropriate amounts of the test material to achieve the nominal concentrations desired. The solution mixtures were stirred (<10% vortex) at room temperature for approx. 24 hrs and allowed to settle for approx. 1 hr before the WAF solutions were removed through an outlet at the bottom of the aspirator bottle. Initial Cell Conc.: 1 x 10^4 cells/mL No. of replicates: 3 replicates /treatment WAF Concentrations (nominal): 0 (untreated controls), 62.5, 125, 250, 500 and 1000 mg/L Physical Measurements: pH was determined at 0 and at 72 hrs. Observations: Cell density was determined for each replicate at 24, 48 and 72 hr by using a hemacytometer. WAF solutions were taken at 0 and 72 hrs and analyzed by TOC.
Results/Remarks	WAF Nominal conc. / % Inhibition (0-72h) (relative to control) Loading Level (mg/L) — Growth — Growth Rate 62.5 — 6.0 % — 4.9 % 125 — -9.3 * — -0.9 250 — -0.59 * — 2.8 500 — -96 *.8 — -19* 1000 — 14 — 8.7 * indicates a stimulatory effect Algal inhibition was not significantly apparent except at the highest nominal WAF concentration of 1000 mg/L, which showed a 14% inhibition (based on growth) and 8.7% inhibition (based on growth rate). The 72-hr NOEC was considered to be at 1000 mg/L WAF (nominal conc.). TOC analyses indicated that the test material was present in the WAF solutions but carbon analysis concentrations were very low (Day 3 detectable values ranging from 0.4 to 3.4 mg/L). The test material has a previously determined water solubility of 0.41 mg/L.
Conclusion	The 72-hr EC_{50} or 72-hr EL_{50} was expected to be >1000 mg/L WAF (nominal concentration) in which the measured TOC was 3.4 mg/L (relative to controls). The 72-hr NOEC was 1000 mg/L WAF. Hence, data indicate that the test material is not expected to cause inhibition to alga at or close to its maximal water solubility limit or water saturated limit (WSL).
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: January 13, 2004.

Biodegradation (CAS No. 180788-27-6)

Test Substance **CAS Number** **Remarks**	Hexanedioic acid, mixed esters with C10-rich, C9-C11 alcohols and TMP 180788-27-6 100% Purity.
Method/guideline	OECD Guideline 301F (1993), Ready Biodegradability: Manometric Respirometry Test.
Test type **GLP** **Year**	Aerobic Biodegradation Yes 1996
Test system	Exposure Period: 28 Days Inoculum: Activated Sludge, Domestic Bacterial population was 1 x 10^6 CFU/ml Kinetics: Not Reported Biodegradation Products: Not Reported Analytical Monitoring: Oxygen uptake monitored
Test Conditions	Treatment replicates were prepared by combining glass-distilled water, a mineral substrate, pH buffer, activated sludge and the appropriate test substance. Three replicates of the test material and two replicates of positive control (sodium benzoate) were prepared and evaluated in 1L glass vessels. Two blank controls were also used to subtract background oxygen in the test system. Oxygen consumed by microorganisms from the oxidation of the test substance was continuously monitored using an automated respirometer. Test flasks were continuously stirred for 28 days in the dark. Test temperature was 22 ± 1 °C. The pH was measured at the end of the 28-day study. Concentrations for Test Substance was 73.7 mg/L for test substance. Concentration for Sodium Benzoate (positive control) was 50.52 mg/L
Results	Biodegradation was 65.24% in 28 days (n=3) for the test material. Data indicated that the test material was not readily biodegradable (did not meet "10-day window" criteria). Sodium benzoate, the positive control reference substance, biodegraded to the extent of 93.40% in 28 days and met the "10-day window" criterion for "readily biodegradable" classification. The biodegradation calculation was performed using the respirometry software from the instrument's manufacturer [Co-ordinated Environmental Service (Kent, UK)], the Theoretical Oxygen Demand (ThOD) and the amount of the test substance added. ThOD of the test material was 2.45 and was based upon the elemental analysis of the test substance (69.12% Carbon, 10.2% Hydrogen, and 20.47% Oxygen).
Conclusions	The test substance was not readily biodegradable.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information
Other	Date: January 14, 2004

Acute Oral Toxicity (CAS No. 68130-55-2)

Test Substance	Hexanedioic acid mixed esters with decanoic acid, heptanoic acid, octanoic acid and PE
CAS Number	68130-55-2
Remarks	100% Purity
Method/guideline	EPA acute oral toxicity (798.1175) test guideline
Test type	Acute oral
GLP	Yes
Year	1994
Test system	Species: Sprague-Dawley Sex: Male and females. No. of animals: 10 (5 males/5 females) Weight: 234-264 grams (males) and 191-216 (females) Dosage: Oral gavage, undiluted test substance administered.
Test Conditions	Remarks: A group of five male and female rats were dosed orally, by stomach tube, at a level of 2000 mg/kg of body weight. The animals were observed for a period of 14 days for mortality and signs of systemic toxicity. The animals were necropsied at the end of the observation period.
Results	LD_{50} was >2000 mg/kg
Remarks	All animals survived treatment with test article. Soft stool was the only clinical observation post dosing observed in three animals on day 1. All animals gained body weight. There were no signs of macroscopic postmortem abnormalities at necropsy.
Conclusions	The acute oral LD_{50} for the test substance was >2000 mg/kg.
Data Quality	Reliable without restrictions [Klimisch reliability 1]
References	Unpublished confidential business information.
Other	Date: December 15, 2003

Repeated Dose Toxicity (CAS No. 68130-55-2)

Test Substance	Hexanedioic acid mixed esters with decanoic acid, heptanoic acid, octanoic acid and PE
CAS Number	68130-55-2
Remarks	100% Purity
Method/guideline	EEC test guideline B.9 Number L383A, (except sites were not occluded and residual test material not wiped off).
Test type	28-Day Dermal Toxicity in Rats
GLP	Yes
Year	1994
Species/strain	Rats/Sprague-Dawley
Route of Administ.	Dermal
Duration of test	4-weeks
No. of animals	Six groups of 10 males and 10 females Group 1 (control); Group 2 (125 mg/kg); Group 3 (500 mg/kg); Group 4 (2000 mg/kg); Group 5 (satellite - control), Group 6 (satellite - 2000 mg/kg)
Dose/Conc. Levels	0.0, 125.0, 500.0 or 2000.0 mg/kg/day

Sex	Males and females
Frequency of treatment	5-Days a week for 4-weeks
Control Group	10 males and females for Group 1 and Group 5
Post-exposure observat.	Only for control and high dose animals.
Statist. Methods	Analysis of variance (ANOVA), associated F-test, Dunnett's test or Tukey's multiple range test.
Remarks on Test Conditions	Test article was applied to the clipped backs of four groups of Sprague-Dawley rats. Group 1 and Group 5, each consisted of 10 males and 10 females that were not treated and served as the control groups. Group 2 and 3 consisted of 10 males and 10 females per group who were administered 125 or 500 mg/kg dose of the test article, respectively. Group 4 and Group 6, each consisted of 10 males and 10 females who were administered a dose level of 2000 mg/kg/day. Animals were fitted with Elizabethan collars. Animals were dosed 5 days/week. After 4-weeks of treatment, Group 1, Group 2, Group 3 and Group 4 were euthanized and subjected to necropsy. The remaining Group 5 (untreated) and Group 6 (high dose) animals remained on test, untreated, for an additional two weeks recovery period.
Results	Animals treated with test article exhibited no signs indicative of systemic toxicity. The test material was not visibly irritating to the skin at the exposure site. Animals exposed to test article at 2000 mg/kg/day gained slightly less weight than untreated controls, achieving statistical significance in the male regular group and in the female satellite group. Exposure to the test article had no effect on food consumption. Statistically significant ($p<0.05$) differences were observed between the data from the untreated control groups and the treated groups for 2 of the 13 hematology parameters evaluated at week 5. Segmented neutrophils were statistically significantly increased in the female mid-dose group. A linear relationship was not found between dose and blood level for this parameter. Lymphocytes were decreased in the male treated satellite group at both weeks 5 and 7. When the historical serum reference values were taken into consideration, the mean value for this parameter at weeks 5 and 7 fell within the normal range as defined by the 10th and 90th percentiles of the historical data. Statistically significant ($p<0.05$) differences were observed between the data from the untreated control groups and the groups treated with test article for 2 of the 19 serum chemistry parameters evaluated at week 5. Aspartate aminotransferase was statistically significantly increased in the female low-dose group. A linear relationship was not found between dose and serum level for this parameter. Alanine aminotransferase was increased in the male treated satellite group. When the historical serum reference values were taken into consideration, the mean value for this parameter fell slightly above the 90th percentile of the historical data. This finding was not considered to be biologically significant because the value was only slightly outside the normal range of the historical data and a similar effect was not observed in the other treated groups. One statistically significant difference (decreased albumin in males) was observed between the serum chemistry data from control and treated animals following the 2-week recovery period. This finding, while falling just outside the normal range (4.5-5.0 g/dL) of the historical data, was not considered to be biologically significant because of the small magnitude of the decrease (3%), and this parameter was not affected at week 5. No test material-related findings were observed at the time of necropsy. No significant differences were seen between the absolute and relative organ weight data of the control and treated regular groups. A significant increase was seen in the relative adrenal and brain weights of the females exposed to test material at 2000 mg/kg/day and sacrificed after a two-week recovery period when compared to those of the untreated controls. This difference is attributed to the statistically significant lower final body weights of the treated animals. Microscopically, the test material-related findings were only observed in the treated skin of the rats exposed to the test material at ≥500 mg/kg/day. Generally these findings were very minor and consisted of a dose-related increased incidence and severity of hyperplasia and hyperkeratosis of the epidermis and sebaceous gland hyperplasia. Microscopic evaluation of the satellite animals showed no differences

	between the control and treated animals, indicating complete reversibility after the 2-week recovery period.
Conclusions	In conclusion, a conservative NOEL was established to be 500 mg/kg/day for systemic toxicity.
Data Quality	Reliable without restrictions [Klimisch reliability 1]
References	Unpublished confidential business information.
Other	Date: December 16. 2003

Genetic Toxicity In Vitro (CAS No. 68130-55-2)

Test Substance	Hexanedioic acid mixed esters with decanoic acid, heptanoic acid, octanoic acid and PE
CAS Number	68130-55-2
Remarks	100% Purity
Method/guideline	EPA test guidelines (CFR 40: 798.5265); except that frequency of plating of positive controls and the order of addition of reactants varied.
Type of Study	
Test System	Ames - *Salmonella typhimurium* Mutation Assay
GLP	Bacterial
Year	Yes 1994
Species/Strain	*Salmonella typhimurium* /TA98; TA100; TA1535; TA1537; and TA1538
Metab. Activation	Arochlor 1254 - induced rat liver S9 mixture.
Concentrations	10.0, 3.0, 1.0, 0.3, and 0.1 µL/50 µL in tetrahydrofuran (THF)
Statist. Methods	A mutagenic response was defined as a greater than two-three fold increase in the number of histidine-revertant colonies over the concurrent vehicle control value.
Remarks on Test Conditions	Concurrent positive control materials were 2-aminoanthracene, 9-aminoacridine, nitrofluorene, and N-methyl-N-nitro-nitrosoguanidine (MNG). The spontaneous reversion frequency for each strain was determined from concurrent untreated and solvent (THF controls. For test material evaluation, fresh bacterial stocks were exposed to graded doses of the test substance both in the presence and absence of exogenous metabolic activation mixture. Revertants were scored 72 hours after exposure. A toxicity pretest was conducted to determine the high dose level (10 µL /plate).
Results	Negative
Remarks	The test substance was negative in all strains. No mutagenic activity was observed over a range of doses from 0.1 to 10 µL /plate with or without metabolic activation. The positive and negative controls gave responses as expected.
Conclusions	The test substance was negative for mutagenic activity (with an independent repeat) with or without metabolic activation.
Data Quality	Reliable without restrictions [Klimisch reliability 1]
References	Unpublished confidential business information.
Other	Date: December 16, 2003

Genetic Toxicity In Vitro (CAS No. 68130-55-2)

Test Substance	Hexanedioic acid mixed esters with decanoic acid, heptanoic acid, octanoic acid and PE
CAS Number	68130-55-2
Remarks	100% Purity
Method/guideline	OECD 473
Type of Study	Chinese hamster ovary (CHO) cell assay
Test System	Mammalian cell
GLP	Yes
Year	1994
Species/Strain	Chinese Hamster Ovary (CHO) cells.
Metab. Activation	With and without Arochlor-induced rat liver S9 mixture.
Concentrations	0.0063 to 0.4 μL/mL (with metabolic activation and without).
Control Groups	Mitomycin C and cyclophosphamide monohydrate were used as a positive control in the assays without S9 activation. The concurrent negative control was the vehicle (acetone).
Statist. Methods	Fisher Exact Probability test and Cochran-Armitage trend test
Remarks on Test Conditions	The preliminary assay indicated that 0.4 μL/mL, which is at or above the limit of solubility in medium for test article, was not cytotoxic and was chosen as the high dose concentration for the main study.
Results	The main metaphase analysis (chromosomal aberration assay) analyzed cells treated at 0.1, 0.2, and 0.4 μL/mL dose levels. No significant increase in the proportion of cells with chromosomal aberrations compared to solvent (acetone) controls occurred with exposure to test articel.
Remarks	An independent repeat assay was also conducted.
Conclusions	Test material did not induce chromosomal damage in this cytogenetic test.
Data Quality	Reliable without restrictions [Klimisch reliability 1]
References	Unpublished confidential business information.
Other	Date: December 16, 2003

Acute fish toxicity (CAS No. 68130-55-2)

Test Substance	Hexanedioic acid mixed esters with decanoic acid, heptanoic acid, octanoic acid and PE
CAS Number	68130-55-2
Remarks	Purity was 100%
Method/guideline	OECD 203; EC L 251/146-154. C.1 (1984)
Type (test type)	Acute fish toxicity study
Test System	Fish, freshwater
GLP	Yes
Year	1992
Species/Strain	Fish: Rainbow trout (*Oncorhynchus mykiss)*

Analyt. Monitoring	No analysis of water samples were performed
Exposure period	96 hours
Statist. Methods	Binomial probability analysis (Stephan *et al.*, 1978)
Test Conditions	96-hr static acute fish toxicity test at five nominal concentrations from 99 mg/L to 5017 mg/L Species: Rainbow trout (*Oncorhynchus mykiss*), mean length 25-26 mm Test performed in 40 L glass vessels containing 30 L well water (hardness 203 mg/L $CaCO_3$); 12.0-13.0 °C; 16 h light/8h dark cycle; unfed; loading 0.26-0.33 g/L. The test substance (oil) was maintained as oil in water dispersion/suspension by a propeller (protected against the fish) above the system which created a vortex on the water surface. No. of fish: 20/treatment Concentrations (nominal): 0 (untreated controls), 99, 493, 1015, 2001 and 5017 mg/L Physical Measurement: The pH, temperature and dissolved oxygen were performed daily. During course of 96 hr study, the mean pH ranged from 7.83-7.90, dissolved oxygen ranged from 8.5 to 8.8 mg/L and the temperature was 12.0-13.0°C. Observations: Mortality/symptoms at 0, 24, 48, 72 and 96 hr
Result	Nominal test conc. Loading Level (mg/L) — Mortality (96-hr) 0 Control (untreated) — 0 99 — 0 493 — 0 1015 — 0 2001 — 0 5017 — 5 No mortality was observed in the fish at any of the nominal concentrations which ranged from 99 mg/L to 5017 mg/L.
Conclusion	The 96-h LC_{50} was > 5017 mg/L (nominal concentration, oil in water suspension/dispersion). Analyses to determine actual concentrations of the test material were not performed. The nominal test concentrations in exposure samples were all above the water solubility of the test material (calculated to be 3.5×10^{-7} mg/L, using EpiWin). Hence, the ecotoxicity data indicate that the test material would not be expected to cause acute toxicity in fish at its water saturation limit or water solubility limit (WSL).
Remarks	1) The fish were relatively small (25-26 mm) compared to that recommended by EC L 383 A: (60±20 mm). Since small fish may be more sensitive, this may be acceptable in a worst case approach. 2) Because the test substance is not soluble in water, it is kept in suspension by a propeller situated above the water surface, utilizing oil in water dispersion method. 3) The LC50 is determined using the nominal concentration, since test material was water-insoluble. 4) The temperature during the study was at the lower range of temperature recommended (12-13°C versus EC L 383 A recommended 12-17°C).
Data Quality	Reliable with restrictions [Klimisch reliability 2]. No chemical analyses were performed to determine the concentration of test substance in water solutions.
References	Unpublished confidential business information.
Other	Date: January 15, 2004.

Acute toxicity to aquatic invertebrate (CAS No. 68130-55-2)

Test Substance	Hexanedioic acid mixed esters with decanoic acid, heptanoic acid, octanoic acid and PE
CAS Number	68130-55-2
Remarks	Purity was 100%
Method/guideline	OECD 202, EEC Directive 92/69/EEC L383 A
Type (test type)	*Daphnia sp.* , Acute immobilization test
Test System	Freshwater invertebrate
GLP	Yes
Year	1994
Species/Strain	Freshwater invertebrate, *Daphnia magna*
Analyt. Monitoring	Analyses were performed by GC-FID of samples collected at 0 and 48 h for WAF solutions derived from the 0, 324, 1296 and 5076 mg/L (nominal concentration) exposure groups.
Exposure period	48 hours
Statist. Methods	Binomial probability analysis (Stephan *et al.*, 1978): Fisher's exact test
Remarks on Test Conditions	48-hr static immobilization study Species *Daphnia magna*, <24 h old Test was performed at 19.8-20.0°C in 250 mL glass beakers containing 200 mL water solutions (WAF) of hardness 200 mg/L ($CaCO_3$), 16 hr light/8 hr dark cycle, unfed No. of daphnids: 10 /replicate, 2 replicates/treatment Concentrations (nominal): 0 (untreated controls), 324, 648, 1296, 2592 and 5076 mg/L as water accommodated fractions (WAF). Physical measurements: At 0 and 48 hr in all concentrations, pH, dissolved oxygen and temperature were performed; range for pH was 8.22-8.48; dissolved O_2 was above 60% of saturation (7.1-7.9 mg O_2/L); temperature was maintained at 19.8-20.0°C. Observations: Immobility and symptoms at 0, 3, 24 and 48 hr Chemical analyses of test material were carried out by solvent extraction from collected WAF solutions (0 and 48 hr) and quantitated by GC/FID. GC limit of detection of test material was 10 mg/L.
Results	WAF Solution Conc. Nominal load rate (mg/L) — Immobility % (48-hr) 0 Control (untreated) — 5% 324 — 0 648 — 5 1296 — 0 2592 — 0 5076 — 0 GC-FID analysis for WAF solutions gave limited results due to the limit of GC detection (ca. 10 mg/L). However, several samples showed measurable concentrations (11-13 mg/L) slightly above the GC limit of detection. The test material has limited water solubility (calculated to be 3.5×10^{-7} mg/L, using EpiWin).
Conclusions	48-hr EC_{50} was > 5076 mg/L WAF (nominal loading rate). No significant immobilization or adverse symptom was observed in the daphnids at any of the tested WAFs compared with controls. Test material could be detectable in the some of the WAF solutions but was close to the limit of analytical detection. The test material has limited water solubility and would be expected to be close to water-saturated levels (WSL) in the tested solutions. The data suggest that test substance would not be expected to cause immobilization at or close to its water saturation levels or water solubility limits (WSL).
Remarks	WAF is the maximum soluble concentration of the nominal test concentrations after 20 hours of stirring and 4 hours of equilibrating. Only the water phase was used in the definitive test

	solutions.
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Chemical analyses were based on limited number of measured samples and analytical limit of GC detection and quantitation.
References	Unpublished confidential business information.
Other	Date: January 15, 2004.

Acute toxicity to aquatic plants (e.g., algae) (CAS No. 68130-55-2)

Test Substance	Hexanedioic acid mixed esters with decanoic acid, heptanoic acid, octanoic acid and PE
CAS Number	68130-55-2
Remarks	Purity was 100%
Method/guideline	OECD 201, EEC L383A/179-186 C3 (1992)
Type (test type)	Algae, growth inhibition test
Test System	Aquatic plant (e.g., algae)
GLP	Yes
Year	1994
Species/Strain	Green algae / *Selenastrum capricornutum*
Analyt. Monitoring	Analyses were performed by GC-FID on samples collected at 0 and 72 hr for WAF solutions from the 0, 324, 1296 and 5076 mg/L (nominal concentration) exposure groups.
Exposure period	72 hours
Statist. Methods	Fischer's exact test and binomial probability analysis
Test Conditions/ Remarks	Static 72 hr algae growth inhibition study Species: Green algae (*Selenastrum capricornutum*) Tests were performed in 250 mL flasks containing 100 mL of algal medium (pH 7.5 ± 0.1); temperature: 24±1°C; continuous illumination (~5000 lux); continuously shaken at 100 rpm Initial Cell Conc.: 1 x 10^4 cells/mL No. of replicates: 3 per treatment Concentrations (nominal): 0 (untreated controls), 324, 648, 1296, 2592 and 5076 mg/L as water accommodated fractions (WAF) prepared at nominal loading rates Physical Measurements: The pH and temperature were performed. The range of pH was reported to be within 7.5 ± 0.1 in flasks; temperature maintained at 24±1°C. Observations: Cell density at 72 hr by counting with hemacytometer Chemical analyses of test material were carried out by solvent extraction from the collected WAF solutions and quantitated by GC/FID. GC limit of detection of test material was 10 mg/L.
Results	WAF Solution Conc. / Nominal load rate (mg/L): 0 Control (untreated); At 72 hr Mean Cell Density: 1.06 x 10^5 cells/mL; Growth Rate % Inhibition: — 324: 5.52 x 10^4; 47.96 % 648: 6.38 x 10^4; 39.90 1296: 4.55 x 10^4; 57.10 2592: 1.46 x 10^4; 86.27 5076: 1.31 x 10^4; 87.67
Remark/comment	1) WAF is the maximum soluble concentration of the nominal test concentrations after 20 hours of stirring and 4 hours of equilibrating. Only the water phase was taken and tested. 2) The analytical results indicate that test material in WAF solutions were below the 10 mg/L detection limit of the GC analytical method. The test material has limited water solubility (calculated to be 3.5 x 10^{-7} mg/L, using EpiWin). 3) Light intensity used was lower than recommended in the OECD 201 guideline. The test is still acceptable, since no effects on the cell growth were seen in the controls.

Conclusions	72-hr EC_{50} was estimated to be 974 mg/L WAF (nominal loading rate) The GC limit of detection in the WAF solutions was below the water solubility limit of the test material. The test substance has limited water solubility and would be expected to be close to water-saturated levels (WSL) in the tested solutions. The data suggest that test substance would not be expected to cause immobilization at or close to its water saturation levels or water solubility limits (WSL).
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Chemical analyses were based on limited number of measured WAF samples and analytical limit of GC detection in the WAF solutions was clearly below the WSL of test material. Percent inhibition based on AUC (area under curve) was not reported.
References	Unpublished confidential business information.
Other	Date: January 15, 2004

Biodegradation (CAS No. 68130-55-2)

Test Substance **CAS Number** **Remarks**	Hexanedioic acid mixed esters with decanoic acid, heptanoic acid, octanoic acid and PE 68130-55-2 Purity was 100%
Method/guideline	EPA 560/6-82-003 (equivalent to OECD 301B methodology) Shake Flask Aerobic Biodegradation - CO_2 evolution method using non-acclimated inoculum
Test type **GLP** **Year**	Aerobic Biodegradation - CO_2 evolution method Yes 1994
Test system	Exposure Period: 28 Days Inoculum: Activated Sludge, Domestic, Unacclimated. Kinetics: Not Reported Biodegradation Products: Not Reported Analytical Monitoring: CO_2 evolution monitored in traps containing base solution.
Test Conditions	Inoculum: Activated sludge obtained from wastewater treatment plant. Volume of inoculum (10.3 ml) added was sufficient to provide a final inoculum solids conc. of 30 mg solids/L. Duplicate flasks Treated [medium + inoculum + test material (10 mg C/l)]; Duplicate flasks Treated [medium + inoculum + test material (20 mg C/l)]; Duplicate flasks Positive Control [medium + inoculum + sodium benzoate (20 mg C/l))]; Duplicate Blank Control [medium + inoculum]. Incubation was performed under continuous shaking in 2L flasks, containing 1L of medium, test substance and/or inoculum at 26-27 ^{0}C in the dark. Evolved CO_2 was collected in appropriate trap containing 10 ml 0.2N KOH. CO_2 was monitored at various time points over a period of 28 days. Flask CO_2 traps were sampled at days 1, 3, 6, 10, 14, 21 and 29. One day prior to the final sampling on day 28, the medium was acidified with 1 ml of concentrated sulfuric acid. The amount of CO_2 was determined in the traps by back titration with 0.2N HCl, after addition of $Ba(Cl)_2$ and indicator. Blank controls were used to subtract for background CO_2 production. Concentrations for Test Substance was 10 mg C /L and 20 mg C/L for test substance. Concentration for sodium benzoate (positive control) was 20 mg C/L.

Results	**Biodegradation Results:**

% Biodegradation [% of ThCO2] mean value

Day	1	3	6	10	14	21	28
Test Material (10 mg C/L)	1.0	21.5	43.9	61.5	72.2	79.1	84.2
Test Material (20 mg C/L)	2.1	20.2	45.0	60.0	75.0	81.5	85.4
Positive Control (sodium benzoate 20 mg C/L)	15.9	48.8	71.0	78.3	81.2	83.0	84.2

The test material met the "10-day window" criteria for ready biodegradability. Positive controls achieved 84.2% biodegradation in 28 days and met the "readily biodegradable" criteria.

Conclusions

Biodegradation was 84.2-85.4% in 28 days. The test substance was readily biodegradable.

Data Quality

Reliable without restrictions [Klimisch reliability 1]. Test method used was essentially equivalent to OECD 301B test method. Temperature was carried out at ambient temperature.

References

Unpublished confidential business information

Other

Date: January 14, 2004

Pentaerythritol esters of isooctanoic and C8-10 fatty acids (No CAS Number)

Melting Point, Boiling Point, Vapor Pressure, Partition Coefficient, Water Solubility (CAS No. -not assigned) Pentaerythritol esters of isooctanoic and C8-10 fatty acids - Surrogate Polyol Ester

Test Substance **CAS Number** **Remarks**	Pentaerythritol esters of isooctanoic and C8-10 fatty acids None assigned yet Purity was 100%		
	GLP (Yes/No)	**METHOD/ GUIDELINE**	**RESULTS / CONCLUSIONS**
Physicochemical Properties			
Melting Point/ Pour Point	Yes	OECD 102	< -40 °C
Boiling Point	Yes	OECD 103	> 300 °C (not determinable, decomposes at temp above 300°C without boiling)
Vapor Pressure	Yes	OECD 104	4.0 x 10^{-6} Pascals at 25 °C
Partition Coeffic.	Yes	OECD 107	log P > 8
Water Solubility	Yes	OECD 105	0.06 mg/L (GC analysis)
Year	1995		
Remarks	Determination of a complete battery of physicochemical properties for the test substance, "Pentaerythritol esters of isooctanoic and C8-10 fatty acids" including those designated above has been carried out under GLP and by methods, which are in compliance with the OECD and EEC Commission Directive 92/69/EEC guidelines. These physicochemical properties determination studies were performed at Pharmaco-LSR Ltd. (now Huntingdon Life Sciences Ltd.), Suffolk, United Kingdom.		
Data Quality	Reliable without restrictions [Klimisch reliability 1].		
References	Unpublished confidential business information.		
Other	Date: January 14, 2004		

Acute Oral Toxicity (PE esters of isooctanoic and C8-10 fatty acids)

Test Substance **CAS Number** **Remarks**	Pentaerythritol esters of isooctanoic and C8-10 fatty acids None assigned yet 100% Purity
Method/guideline	OECD 401 (1987)
Test type **GLP** **Year**	Acute oral toxicity Yes 1995
Test system	Species: Rats (Crl:CDBR strain), approx. 8-9 weeks old Sex: Male and females. No. of animals: 10 (5 males/5 females) Weight: 225-243 gm (males) and 184-201 gm (females) Route: Oral gavage, undiluted test substance administered Dosage: 2000 mg/kg body weight (limit dose) Statist. Meth.: Not applicable.
Test Conditions	A group of five male and female rats (fasted overnight) were dosed orally, by stomach intubation, at a level of 2000 mg/kg of body weight. Clinical observations were performed at 1, 2, 4 and 6 hrs after dose administration and daily thereafter over a period of 14 days. The animals were observed daily for a period of 14 days for mortality and signs of systemic toxicity. Body weights were recorded on days 0, 7 and 14. The animals were sacrificed and necropsied at the end of the observation period on day 14.
Results/Remarks	All animals survived treatment with test substance and gained weight over their initial (Day 0) values and were free of gross abnormalities at postmortem examination. Reported clinical observations were limited to two females with anogenital staining at the 4 or 6 hr observation interval on Day 0. Oral dose of test material did not produce any consistent signs of systemic toxicity and all animals were free of observable abnormalities throughout the study.
Conclusions	The acute oral LD_{50} was >2000 mg/kg for the test substance.
Data Quality	Reliable without restrictions [Klimisch reliability 1]
References	Unpublished confidential business information.
Other	Date: January 15, 2004

Repeated-Dose Toxicity (PE esters of isooctanoic and C8-10 fatty acids)

Test Substance	Pentaerythritol esters of isooctanoic and C8-10 fatty acids
CAS Number	None assigned yet
Remarks	100% Purity
Method/guideline	OECD 407
Test type	28-Day oral toxicity study in rats
GLP	Yes
Year	1994
Species/strain	Rats /Crl:CD BR, age approximately 7 weeks, weight 216 to 251 g (males), 161.9 to 187.8 g (females)
Route of Administ.	Oral gavage
Duration of test	Twenty-eight (28) days.
No. of animals	25 males and 25 females; 5/sex/dose level
Dose/Conc. Levels	0 (carrier control), 100, 500 and 1000 mg/kg/day of test substance in PEG 400. In addition, a positive control (20 mg/kg of acrylamide in PEG 400) group was included.
Sex	Male and female
Frequency of treatment	Daily oral administration, 7 days/week for 4 weeks (28 days)
Control Group	Yes. Carrier (polyethylene glycol; PEG 400) control group. In addition, a positive control (20 mg/kg of acrylamide in PEG 400) group was included
Statist. Methods	Bartlett's test, ANOVA, Dunnett's test, Kruskal-Wallis test, Jonckheere's test, Dunn's Summed Rank test
Test Conditions/ Remarks	This study was conducted to evaluate the potential of the test substance to cause cumulative toxicity and neurotoxicity when administered orally by gavage to rats for a period of 28 days. Three groups of five male and five female rats were administered the test substance/carrier mixtures at dose levels of 100, 500, and 1000 mg/kg/day. Additionally a group of five male and five female rats served as a control and received carrier (PRG400). Additionally a group of five male and five female rats served as positive control and received 20 mg/kg of acrylamide in carrier (PRG400). Dosing volume levels were adjusted weekly based on the most recent body weights. Neurotoxicity was evaluated by assessments of Functional Observational Battery (FOB) and motor activity. Clinical observations were made daily throughout the study. A complete functional observational battery was conducted on all animals prior to receiving test material and during Week 1 and 4 of dosing. There were single or low occurrences of several FOB parameters in test substance treated animals at both the Day 8 and 27 observation interval, but in the absence of a clear consistent pattern of response, these observations were considered incidental and unrelated to treatment. Treatment-related effects were observed in the acrylamide animals and included increased foot splay, decreased hindlimb strength, decreased muscle tone, gait impairment, increased landing foot splay. Typical effects of peripheral neuropathy were expected in the acrylamide group. Body weights were recorded pretest, at dose initiation (Day 0), and on Days 7, 14, 21 and 27 for all animals. Food consumption was measured weekly during the test period. Hematology, serum chemistry, and coagulation studies were performed on all animals on Day 28. A full macroscopic postmortem examination was performed on all animals and required organs were preserved. Selected organs were weighed at study termination. A range of tissues was examined microscopically.

Results	There were no statistically significant differences observed for the functional observational battery parameters or motor activity. All animals displayed increases in body weight over their initial values. There were no biologically significant differences in mean body weight or mean food consumption between treated and control animals at any interval. In conclusion, oral administration of the test substance to rats by gavage did not produce signs of overt systemic toxicity at any dose level tested. There were no treatment-related clinical in-life, functional observation battery, or gross postmortem or microscopic findings; no treatment-related mortality; and no adverse effects on body weight, food consumption, clinical laboratory parameters, or organ weights. Histomorphologic observations of ovaries and testis in treated animals were reported to be normal.
Conclusions	Therefore, a No Observable Adverse Effect Level (NOAEL) for the test substance was established at 1000 mg/kg/day
Data Quality	Reliable with restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: January 16, 2004

Genetic Toxicity in Vitro (PE esters of isooctanoic and C8-10 fatty acids)

Test Substance **CAS Number** **Remarks**	Pentaerythritol esters of isooctanoic and C8-10 fatty acids None assigned yet 100% Purity .
Method/guideline **Type of Study** **Test System** **GLP** **Year**	OECD 471 (1983); EC 67/548/EEC Annex V. Part B.14 (1993) Bacterial Reverse Mutation Assay Bacterial (*Salmonella typhimurium*) Yes 1995
Species/Strain **Metab. Activation** **Concentrations** **Statist. Methods**	*Salmonella typhimurium* / TA98, TA100, TA1535, TA1537 , TA 1538 Aroclor 1254-induced rat liver preparations (S9 mixture) Test concentrations: 50, 100, 500, 1000 and 5000 µg/plate Mean revertant colony count and std deviation (Snedor and Cochran, 1989)
Test Conditions/ Remarks	Negative control: acetone or DMSO (vehicle) Positive controls: 2AA (all strains with S9), 2NF (TA98, TA 1538 without S9), MNNG (TA100, TA1535 without S9), 9AA (TA1537 without S9). Abbrev. 2AA (2-Aminoanthracene); 2NF (2-Nitrofluorene); MNNG (N-Methyl-N-Nitro-N-Nitrosoguanidine); 9AA (9-Aminoacridine); Procedure: There were 3 plates /dose groups/treatment. Samples of bacteria (0.1 mL), followed by vehicle (acetone or DMSO) (100 µL), appropriate test substance dilution (100 µL) or appropriate positive control substance dilution (100 µL), and 0.5 mL of S9 mix (+S9) or saline (-S9), were added to sterile glass test tubes containing molten top agar. The mixture was vortexed and immediately poured on plates containing a layer of minimal agar medium. After the top agar solidified the plates were inverted and incubated at 37 ± 2 °C for approximately 2 days. All plates were evaluated after approximately two days of incubation for gross toxic

	effects and total revertant colony numbers. Revertant colonies were counted via a Biotran III Colony Counter. Two positive controls, a non-treated control and two vehicle controls were tested concurrently for each strain. The vehicle and positive controls were tested using a 100 µL sample.
Results/Remarks	The test material did not induce significant increases in mean revertant colonies (equal to or greater than two or three times the vehicle control) or toxicity in tester strains TA98, TA100, TA1535, TA1537 or TA 1538 at any dose level tested with or without metabolic activation in the assays. Beading of the test substance (a common finding with materials having low water solubility) was observed in the assays in all bacteria strains at doses equal to or greater than 500 µg/plate, with or without metabolic activation. The test substance was negative for mutagenic activity in the five *Salmonella* tester strains, with or without metabolic activation. No mutagenic activity was observed at concentrations tested. The positive controls gave the appropriate responses as expected.
Conclusions	The test substance was not mutagenic, with or without metabolic activation in the *Salmonella*/Mammalian Microsome Reverse Mutation assay.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: January 15, 2004.

Genetic Toxicity in Vitro (PE esters of isooctanoic and C8-10 fatty acids)

Test Substance **CAS Number** **Remarks**	Pentaerythritol esters of isooctanoic and C8-10 fatty acids None assigned yet 100% Purity .
Method/guideline	OECD 473 (1983)
Type of Study **Test System** **GLP** **Year**	Chinese hamster ovary (CHO) cell assay , in vitro mammalian cytogenetic test Mammalian cell Yes 1995
Species/Strain **Metab. Activation** **Concentrations**	Chinese Hamster Ovary (CHO) cells. With and without Arochlor-induced rat liver S9 mixture. 5, 10, 20, 40, 80 and 160 µg/mL (initial assay) and 40, 80 and 160 µg/mL (repeat assay) . These concentrations were selected based on the results of a toxicity pretest. The test substance was dissolved in acetone
Control Groups	Positive Controls were either 9,10-Dimethyl-1,2-benzanthracene [DMBA](+S9), or 1-Methyl-3-Nitro-1-Nitrosoguanidine [MNNG] (-S9). The concurrent negative control was the vehicle (acetone).
Statist. Methods	Fisher Exact Probability test, permutation test for dose-related trends
Test Conditions/ Remarks	The definitive study consisted of two phases: an initial chromosomal aberration assay with a 16 hour harvest time, and a repeat assay with both 16 and 40 hour cell harvest times. Test concentrations were selected based on solubility, cell confluency (survival) and the percentage of mitotic cells. The CHO cells were cultured in McCoy's 5A Medium containing 10% fetal bovine serum and 2 mM L-glutamine at 37±2°C, in 4-6% CO_2 in air. Two sets of duplicate cultures were prepared; one set was treated with the test substance with activation and the other was treated with the test

	one set was treated with the test substance with activation and the other was treated with the test substance without activation. Each flask received a 50 µL sample of the test substance mixture, positive control mixture, DMBA or MNNG, or vehicle (acetone). Flasks with (+S9) metabolic activation were treated for 3 hrs. The cultures were incubated to their respective harvest times (16 or 40 hours). A spindle inhibitor was added to the flasks approximately 2-3 hours prior to harvest to arrest the cells in c-metaphase. The cells were harvested and slides prepared to evaluate chromosomes. The positive control materials were evaluated for chromosomal aberrations at the 16 hour harvests only.
Results/Remarks	There were no statistically significant differences in the number of chromosomal aberrations between treated and vehicle control groups in either the initial or repeat assays at any dose concentrations evaluated (40, 80 and 160 µg/mL), either with or without metabolic activation. The positive controls (i.e., DMBA and MNNG) and vehicle controls performed in an appropriate manner as expected. The test material did not cause any biologically significant increases in chromosomal aberrations in this study.
Conclusions	The test material did not induce chromosomal aberrations in Chinese hamster ovary (CHO) cells.
Data Quality	Reliable without restrictions [Klimisch reliability 1]
References	Unpublished confidential business information.
Other	Date: January 15, 2004

Genetic Toxicity In Vivo (PE esters of isooctanoic and C8-10 fatty acids)

Test Substance	Pentaerythritol esters of isooctanoic and C8-10 fatty acids
CAS Number	None assigned yet
Remarks	100% Purity
Method/guideline	OECD 474 (1983)
Type of Study	*In vivo* micronucleus assay
Test system	Bone marrow and peripheral blood cells
GLP	Yes
Year	1995
Species/Strain	Mouse / CD-1, approx. 9 weeks old
Sex	25 male and 25 female
No. of animals	5 animals/dose for three doses, vehicle (peanut oil) control and positive control (cyclophosphamide, 20 mg/kg in water)
Route of Administ.	Oral gavage (diluted in peanut oil vehicle)
Doses/conc. levels	500, 1000 and 2000 mg/kg
Exposure period	Three single oral doses administered approx. 24 hrs apart.
Controls	Vehicle carrier control and positive control (cyclophosphamide, 20 mg/kg)
Statist. Methods	ANOVA, Duncan's multiple range test, Wilk's criterion or the Kolomogorov-Smirnov statistics test, Kruskal-Wallis one-way ANOVA, Dunn's summed rank test, Jonkheere's test of ordered response.
Test Conditions/	Prior to the start of the assay, a range-finding study was performed. Based on the results of the range finding study, the test substance was administered via oral gavage to groups of 5 male and 5 female mice at doses of 500, 1000, and 2000 mg/kg. A fourth group of mice served as a carrier control and received peanut oil only. A fifth group served as a positive control and received an oral dose of 20 mg/kg of cyclophosphamide in water. Dose volumes for all groups did not exceed 1 ml/100 gm b.w. The test material was administered in three treatments approximately 24 hours apart.

	Clinical observations were made after each test substance administration and prior to terminal sacrifice. Body weights were recorded before testing, on the first day of dosing All animals were sacrificed approximately 24 hours following the last test substance administration. Immediately after sacrifice, both femurs were removed from each animal and processed. Bone marrow smears were prepared, 2 slides per animal, and stained using acridine orange. Two thousand polychromatic erythrocytes (PCEs) from each animal were examined for the presence of micronuclei and the percent of polychromatic erythrocytes in the total population of erythrocytes was determined. All animals survived to scheduled study termination and were free of clinical signs throughout the study.
Remarks	This study was conducted in order to evaluate the potential of the test substance to induce micronucleated polychromatic erythrocytes (MNE) in the bone marrow in CD-1 mice. The *in vivo* mammalian bone marrow micronucleus assay is a short term test to evaluate the clastogenic (chromosome breaking) potential of test materials. Evidence of chromosome breakage or nondisjunction can be readily detected as MNEs.
Results	There were no dose-related increases or statistically significant differences in micronuclei formation at any dose level of the test material evaluated. Cytotoxicity was not observed since there were no statistically significant decreases in the percentage of polychromatic erythrocytes when compared with carrier control. The positive control substance induced a statistically significant increase in the mean number of MNE which indicated that cyclophosphamide) was clastogenic and responded in an appropriate manner. In addition, the positive control substance induced cytotoxicity.
Conclusions	The test material did not produce any increase in micronuclei formation and did not induce cytotoxicity in the bone marrow of CD-1 mice. Hence, the test material was considered negative in the mouse bone marrow micronucleus test under the conditions of the study.
Data Quality	Reliable without restrictions [Klimisch reliability 1]
References	Unpublished confidential business data.
Other	Date: January 16, 2004

Reproductive Toxicity /Developmental Toxicity (PE esters of isooctanoic and C8-10 fatty acids)

Test Substance	Pentaerythritol esters of isooctanoic and C8-10 fatty acids
CAS Number	None assigned yet
Remarks	100% Purity
Method/guideline	Official Journal of the European Communities L133, Methods for Determination of Toxicity, "Teratogenicity" (Annex V, adopted November 18, 1987) and the EPA TSCA test guidelines for developmental toxicity studies (40 CFR, Part 798).
Test type	Developmental Toxicity Study in Rats
GLP	Yes
Year	1994
Species/strain	Rats/Crl:CD BR VAF/Plus (9-10 weeks old)
Route of Administ.	Oral
Duration of test	21-Days
No. of animals	Four groups of 25 pregnant rats
Dose/Conc. Levels	0 (carrier control), 100.0, 500.0 and 1000.0 mg/kg/day
Sex	Females

Frequency of treatment **Control Group** **Post-exposure observat.** **Statist. Methods**	Daily on each gestation days 6-15 25 Females for Group 1 (Carrier Control: Polyethylene glycol-PEG 400) None. Bartlett's Test; Cochran's transformation; Dunnett's test; Kruskal-Wallis Test; Fisher Exact Test; Armitage's Test
Remarks on Test Conditions	Males and females were paired and housed overnight until confirmation of mating [sperm/plug = Gestation Day (GD)]. Each mated female then was returned to its own cage and new females were placed in the males' cages until the required number of mated females was obtained. Mated females were assigned to dose groups in the order of mating. Clinical observations were made daily during gestation. The animals were examined for viability at least twice daily during the treatment period and at least once daily at other times during the study. Body weight and food consumption measurements were made on GD 0, 6, 9, 12, 15, 18, and 21. On GD 21, animals were euthanized and cesarean sections were performed. Gross necropsies were performed, uterine weights with ovaries attached were measured, uterine contents were examined, and the required uterine implantation data were recorded. All live fetuses were weighed, sexed externally, and examined externally for gross malformations. Approximately one-half of the fetuses of each litter were decapitated after being euthanized. The heads were preserved in Bouin's solution for at least two weeks, rinsed, and subsequently stored in 70% alcohol. Sections of the fetal heads were prepared with a razor blade, were examined for the presence of abnormalities and then discarded. The viscera of these fetuses were immediately examined for abnormalities by dissection. The remaining one-half of the live fetuses were eviscerated, processed for skeletal staining, and examined for presence of malformations and ossification variations.
Results	There was no treatment-related mortality. Unscheduled mortality was limited to one mid-dose female due to a dosing error. There were no treatment-related clinical signs observed in the animals during gestation and the majority of dams were free of observable abnormalities during the entire gestation period. Soft stool was observed in both the treated and control animals following dose initiation. Thus, soft stool was considered a response to the carrier rather than a toxicity effect. All animals displayed increases in body weight over their initial values. There were no statistically significant differences in mean body weight, mean uterine weight, mean body weight change, mean corrected body weight, or mean food consumption between treated and control animals at any interval. There were no postmortem findings which were judged to be the result of treatment. There were no statistically significant differences in mean uterine implantation parameters between treated and control groups. There were no biologically and/or statistically significant differences in mean fetal body weight or mean skeletal ossification sites between treated and control fetuses. Similarly, there were no statistically significant differences in total or individual variations or malformations (external, visceral, or skeletal) in the treated groups when compared with controls on either a per fetus or per litter basis.
Conclusions	The maternal and developmental NOAELs were established at 1000 mg/kg. There was no evidence of maternal toxicity observed at any dose level tested. There were no statistically significant differences in mean body weight, body weight change, uterine weight, corrected body weight, food consumption, or uterine implantation data Between treated and control animals. Additionally, there was no mortality or adverse clinical/postmortem signs which were considered treatment-related. In the fetuses, there was no evidence of growth retardation or increased fetal death in the treated groups compared with controls. Additionally, there were no biologically significant differences in total or individual variations or malformations (external, visceral, or skeletal) in the treated groups when compared with controls on either a per fetus or per litter basis. The

	test material was not considered embryotoxic nor teratogenic under the conditions of this study. Based on the data, the maternal and developmental NOAELs were established at 1000 mg/kg.
Data Quality	Reliable without restrictions [Klimisch reliability 1]
References	Unpublished confidential business information
Other	Date: January 16, 2004

Acute fish toxicity (PE esters of isooctanoic and C8-10 fatty acids)

Test Substance	Pentaerythritol esters of isooctanoic and C8-10 fatty acids
CAS Number	None assigned yet
Remarks	100% Purity
Method/guideline	OECD 203 (1992), 67/548/EEC, Annex V, Part C.1 (1993)
Type (test type)	Acute fish toxicity study
Test System	Fish, freshwater
GLP	Yes
Year	1994
Species/Strain	Fish: Fathead minnow (*Pimephales promelas)*
Analyt. Monitoring	Analyses water solutions were performed by GC-FID
Exposure period	96 hours
Statist. Methods	Not indicated
Test Conditions	96-hr semi-static (renewal) acute fish toxicity test was carried out with water solutions of the test material at five nominal concentrations ranging from 0.312 mg/L to 5.0 mg/L. Ethanol was used as a vehicle to help solubilize the test material into solution in the preparation of the test solutions. Species: Fathead minnow (*Pimephales promelas)*, mean length 60 ± 10 mm Test performed in glass culture dishes (with minimum headspace) containing 0.65 L of the water solutions prepared from laboratory dilution water (hardness 242-252 mg/L $CaCO_3$); 22.8-24.0°C; 16 h light/8h dark cycle; unfed; mean loading 0.061 g/L. Preliminary experiments were carried out which showed that approx. 5.0 mg/L was the maximum achievable water-soluble conc of the test material using ethanol as vehicle. The water test solutions were prepared in containers containing laboratory dilution water and the appropriate amounts of the test material (using stock solutions of 50 mg/ml test material in ethanol) to achieve the nominal concentrations desired. The mixtures were stirred at room temperature for 2-3 minutes and the water-accommodated fraction of each treatment was divided into two replicate test chambers. No. of fish: 20/treatment with 10/test chamber (duplicate) WAF Concentrations (nominal): 0 (untreated controls), 0 (ethanol vehicle control, < 0.1 ml/L), 0312, 0.625, 1.25, 2.5 and 5.0 mg/L Physical Measurement: The pH, temperature and dissolved oxygen were performed daily. The pH ranged from 7.7 to 7.9. Dissolved oxygen levels remained above 60% saturation for all treatment and temperature ranged from 22.8 to 24.0°C. WAF solutions were taken on 0, 24 and 72 hrs and analyzed by GC-FID for the test material. Observations: Mortality, abnormal behavior and appearance of fish at 24, 48, 72 and 96 hr

Results/Remarks	Water Nominal Conc. Loading Level (mg/L) / Mean Measured (Day 0) Concentration (mg/L) / Mortality (96-hr) 0 (control) ~LOD 0% 0 (vehicle control) ~LOD 0 0.312 0.24 0 0.625 0.85 0 1.25 1.30 0 2.5 2.15 0 5.0 4.11 0 LOD = limit of detection was 0.043 mg/L for GC-FID analysis No mortality was observed in the fish at any of the test solutions during the 96-hr exposure period. GC-FID analyses of WAF solutions indicated that test material was present in the range of 0.24 to 4.11 mg/L. Ethanol appears to help solubilize the test material into the water. The test material has a previously determined water solubility of 0.06 mg/L (no ethanol vehicle).
Conclusion	The 96-hr LC_{50} or 96-hr LL_{50} was > 4.11 mg/L (measured conc.) or > 5 mg/L WAF (nominal concentration) . No mortality was observed at any of the tested WAF concentrations (nominal or measured). Hence, data indicate that the test material not expected to cause mortality in fish at or above its water solubility limit or water saturated limit (WSL).
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: January 16, 2004.

Acute toxicity to aquatic invertebrate (PE esters of isooctanoic and C8-10 fatty acids)

Test Substance	Pentaerythritol esters of isooctanoic and C8-10 fatty acids
CAS Number	None assigned yet
Remarks	100% Purity
Method/guideline	OECD 202 (1984)
Type (test type)	*Daphnia sp.* , Acute immobilization test
Test System	Freshwater invertebrate
GLP	Yes
Year	1995
Species/Strain	Freshwater invertebrate, *Daphnia magna*
Analyt. Monitoring	Analyses of WAF solutions were carried out using Total Organic Carbon (TOC)
Exposure period	48 hours
Statist. Methods	Not indicated
Test Conditions	48-hr static acute immobilization study was carried out with water accommodated fractions (WAFs) of the test material at five nominal concentrations ranging from 62.5 mg/L to 1000 mg/L Species *Daphnia magna*, <24 h old Test was performed at 21.2 ± 0.3°C in 125 mL glass beakers containing sufficient volume (so that there is no headspace) of the water accommodated fraction (WAF) solutions prepared from laboratory dilution water (hardness 190 mg/L $CaCO_3$); 16 h light/8h dark cycle; daylight intensity 59.5-59.7 foot-candles; unfed.; loading was approximately 1 daphnid per 2 mL solution . The WAF solutions were prepared in glass aspirator bottles using 1L of laboratory dilution

	water and the appropriate amounts of the test material to achieve the nominal concentrations desired. The WAF mixtures were stirred (5 to 10% vortex) at room temperature for approx. 24 hrs and allowed to settle for approx. 1 hr before the WAF solutions were removed through an outlet at the bottom of the aspirator bottle. No. of daphnids: 20 /treatment in four replicates (5daphnids/replicate) WAF Concentrations (nominal): 0 (untreated controls), 62.5, 125, 250, 500 and 1000 mg/L Physical Measurement: The pH, temperature and dissolved oxygen measurements were performed on Day 0 and 2. During course of 48-hr study, the pH in WAF solutions ranged from 7.6 to 8.0; dissolved oxygen levels remained clearly above 60% saturation for all treatment (range 7.0-8.3 mg O_2/L), and temperature was 21.2 ± 0.3°C. Limited WAF solutions were taken at 0 and 48 hrs and analyzed by TOC. Observations: Immobilization and symptoms were observed daily.
Results/Remarks	WAF Nominal conc. Loading Level (mg/L) — % Immobility (48-hr) 0 — 0% 62.5 — 0 125 — 0 250 — 0 500 — 0 1000 — 5 After 48-hr exposure period, immobilization was not observed in any of the WAF exposure group except for 5% immobilization at the (highest) 1000 mg/L nominal WAF exposure group. TOC analyses was performed only at 1000 mg/L and provided limited information except to indicate that the test material was present in the water solution at Day 2. The test material has a previously determined water solubility of 0.06 mg/L
Conclusion	The 48-hr EC_{50} or 48-hr EL_{50} was >1000 mg/L WAF (nominal concentration). The test material has a previously determined water solubility of 0.06 mg/L. No immobilization or abnormal behavior was observed at the any of the test concentrations of the WAFs (except 5% at 1000 mg/L nominal exposure group. Hence, data indicate that the test material is not expected to cause immobilization in daphnids at or close to its maximal water solubility limit or water saturated limit (WSL).
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information.
Other	Date: January 16, 2004.

Acute toxicity to aquatic plants (e.g., algae) (PE esters of isooctanoic and C8-10 fatty acids)

Test Substance	Pentaerythritol esters of isooctanoic and C8-10 fatty acids
CAS Number	None assigned yet
Remarks	100% Purity
Method/guideline	OECD 201 (1984)
Type (test type)	Algae, growth inhibition study
Test System	Aquatic plant (e.g., algae)
GLP	Yes
Year	1995

Species/Strain	Green algae / *Selenastrum capricornutm*
Analyt. Monitoring	Analyses of WAF solutions were carried out using TOC analysis
Exposure period	96 hours
Statist. Methods	ANOVA, SAS regression analysis
Test Conditions	96-hr static algae growth inhibition study was carried out with water accommodated fractions (WAFs) of the test material at five nominal concentrations ranging from 62.5 mg/L to 1000 mg/L. Species: Green algae (*Selenastrum capricornutum*) Tests were performed in 125 mL flasks containing approx. 50 mL of WAF-algal medium solutions (pH 7.5 ± 0.1); temperature: 22.8 ± 0.8°C; continuous illumination (~4599-5118 lux); continuously shaken at 100 rpm. Sufficient alga was added to obtain the initial cell count of 1 x 10^4 cells/mL for the experiments. The WAF solutions were prepared in glass aspirator bottles using 1L of algal nutrient medium solution and the appropriate amounts of the test material to achieve the nominal concentrations desired. The solution mixtures were stirred (<10% vortex) at room temperature for approx. 24 hrs and allowed to settle for approx. 1 hr before the WAF solutions were removed through an outlet at the bottom of the aspirator bottle. Initial Cell Conc.: 1 x 10^4 cells/mL No. of replicates: 4 replicates /treatment WAF Concentrations (nominal): 0 (untreated controls), 62.5, 125, 250, 500 and 1000 mg/L Physical Measurements: pH was determined at 0 and at 96 hrs; mean pH was 7.4 on Day 0 and 8.7-9.0 on Day 4. Observations: Cell density was determined for each replicate at 24, 48, 72 and 96 hr by using a Turner filter fluorometer. WAF solutions were taken at 0 and 96 hrs and analyzed by TOC.
Results/Remarks	WAF Nominal conc. / % Inhibition (0-96 h) relative to control Loading Level (mg/L) — Growth Rate — AUC Growth Curve 62.5 — 4.1 % — 20.9 % 125 — 2.7 — 13.2 250 — 3.0 — 16.4 500 — 3.0 — 12.0 1000 — 2.5 — 9.8 Algal inhibition (particularly growth rate) was not significantly apparent for the WAF concentrations tested. The 96-hr NOEC was considered to be at 1000 mg/L WAF (nominal conc.). TOC analyses indicated that the test material was present in the WAF solutions but determinations were limited to the 1000 mg/L WAF solutions. The test material has a previously determined water solubility of 0.06 mg/L.
Conclusion	The 96-hr EC_{50} or 96-hr EL_{50} was expected to be >1000 mg/L WAF (nominal concentration) based on the available data. The 96-hr NOEC was 1000 mg/L (nominal conc.) WAF. Hence, data indicate that the test material is not expected to cause inhibition to alga at or close to its maximal water solubility limit or water saturated limit (WSL).
Data Quality	Reliable with restrictions [Klimisch reliability 2]. Limited analytical results and lack of statistically significant dose response effect over exposure range based on reduction in area under the growth rate or the average specific growth rate.
References	Unpublished confidential business information.
Other	Date: January 16, 2004.

Biodegradation (PE esters of isooctanoic and C8-10 fatty acids)

Test Substance **CAS Number** **Remarks**	Pentaerythritol esters of isooctanoic and C8-10 fatty acids None assigned yet 100% Purity
Method/guideline **Test type** **GLP** **Year**	OECD 301B Modified Sturm, 92/69/EEC L383, C4 Aerobic Ready Biodegradability test (Modified Sturm - CO_2 evolution method) Yes 1995
Test system	Exposure Period: 28 Days Inoculum: Activated sludge from municipal sewage treatment plant, 1 x 10^6 CFU/mL Kinetics: Not Reported
Test Conditions	Inoculum: activated sludge from domestic wastewater treatment plant. Sufficient inoculum was added to each vessel to provide 1 x 10^6 CFU/mL Blank control [medium + inoculum] (n=2) Positive control [medium + inoculum + sodium benzoate (19 mg C/L)] (n=3) Treated [medium + inoculum + test material (18 mg C/L)]. (n=3) Medium was buffered mineral medium solution (initial pH taken) as outline in OECD 301B guidelines. Biodegradation experiments were performed in the dark under continuous stirring in 4 L glass vessels. The inoculum and medium (3 L) were pre-acclimated during 24 hours, and subsequently treated and aerated for 28 days at 20-23°C with CO_2-free air. The outcoming air was passed through 3 consecutive CO_2-traps containing 0.05N $Ba(OH)_2$. The amount of CO_2 was determined in the traps by back-titrating with standardized 0.1N HCl at various time intervals. The pH was measured on day 28 in the individual vessels. Concentrations for Test Substance was 18 mg C /L for test substance. Concentration for sodium benzoate (positive control) was19 mg C/L.
Results	Biodegradation occurred to the extent of 65.05% in 28 days for the test substance. The test substance did not meet the "10-day window" criterion for "readily biodegradable". Positive controls (sodium benzoate) achieved 87.8% biodegradation in 28 days and met the readily biodegradable classification. Biodegradation values were corrected for background CO_2 with blank controls. **Biodegradation Results:**

	% Biodegradation [% of ThCO2]									
Day	**2**	**4**	**6**	**8**	**12**	**15**	**19**	**22**	**26**	**28**
Test Substance	1.6	8.1	14.9	21.7	32.4	44.6	56.4	59.1	61.5	65.0
Positive Control (sodium benzoate)	24.4	53.1	69.3	76.2	82.5	84.6	85.6	85.9	86.9	87.8

Conclusions	The test substance was biodegraded to the extent of 65.0% in 28 days. The test material was not readily biodegradable.
Data Quality	Reliable without restrictions [Klimisch reliability 1].
References	Unpublished confidential business information
Other	Date: January 16, 2004